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JC806 U.S. PTO 08/29/01

JC828 U.S. PTO 09/942515 08/29/01

PTO/SB/05 (08-00)

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Approved for use through 10/31/2002. OMB 0651-0032
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	RDID 00108US
First Inventor	Bhullar
Title	Biosensor
Express Mail Label No.	ET471793701US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

1. [X] Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. [ ] Applicant claims small entity status. See 37 CFR 1.27.
3. [X] Specification [*Total Pages* 18] *(preferred arrangement set forth below)*
   - Descriptive title of the invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. [X] Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 4]
5. Oath or Declaration [*Total Pages* 5]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63 (d)) *(for continuation/divisional with Box 17 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)
6. [ ] Application Data Sheet. See 37 CFR 1.76
7. [ ] CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ paper
   c. [ ] Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. [ ] Assignment Papers (cover sheet & document(s))
10. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
11. [ ] English Translation Document *(if applicable)*
12. [X] Information Disclosure Statement (IDS)/PTO-1449 [X] Copies of IDS Citations
13. [ ] Preliminary Amendment
14. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [ ] Other: .....

17. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:*

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: _____/_____

Prior application information: Examiner _____ Group / Art Unit: _____

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

18. CORRESPONDENCE ADDRESS

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Name (Print/Type)	Jill L. Woodburn	Registration No. (Attorney/Agent)	39,874
Signature	Jill L. Woodburn	Date	August 29, 2001

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FEE TRANSMITTAL for FY 2001

Patent fees are subject to annual revision

TOTAL AMOUNT OF PAYMENT ($) 1006

Complete if Known	
Application Number	To Be Assigned
Filing Date	Herewith (8.29.01)
First Named Inventor	Bhullar
Examiner Name	Unknown
Group Art Unit	Unknown
Attorney Docket No.	RDID00108 US

METHOD OF PAYMENT

1. [X] The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
   Deposit Account Number: 02-2958
   Deposit Account Name: Roche Diagnostics Corporation
   [X] Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
   [ ] Applicant claims small entity status. See 37 CFR 1.27
2. [ ] **Payment Enclosed:**
   [ ] Check [ ] Credit card [ ] Money Order [ ] Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	710	201	355	Utility filing fee	710
106	320	206	160	Design filing fee	
107	490	207	245	Plant filing fee	
108	710	208	355	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 710

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below		Fee Paid
Total Claims	32	-20** =	12	X 18	=	216
Independent Claims	4	- 3** =	1	X 80	=	80
Multiple Dependent					=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	80	209	40	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 296

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	390	216	195	Extension for reply within second month	
117	890	217	445	Extension for reply within third month	
118	1,390	218	695	Extension for reply within fourth month	
128	1,890	228	945	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,240	241	620	Petition to revive - unintentional	
142	1,240	242	620	Utility issue fee (or reissue)	
143	440	243	220	Design issue fee	
144	600	244	300	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	710	246	355	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	710	249	355	For each additional invention to be examined (37 CFR § 1.129(b))	
179	710	279	355	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) --

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Jill L. Woodburn	Registration No. (Attorney/Agent)	39,874	Telephone	812 842-2660
Signature	Jill L. Woodburn			Date	Aug. 29, 2001

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BIOSENSOR

FIELD OF THE INVENTION

The present invention relates to a biosensor, more particularly to an electrochemical biosensor with a code pattern thereon.

BACKGROUND AND SUMMARY OF THE INVENTION

Electrochemical biosensors are known. They have been used to determine the concentration of various analytes from biological samples, particularly from blood. Electrochemical biosensors are described in U.S. Patent Nos. 5,413,690; 5,762,770; 5,798,031; and 5,997,817 the disclosure of each of which is expressly incorporated herein by reference. It is also known to include a code on a test strip that identifies the manufacturing batch of the strip. See WO 99/22236.

According to one aspect of the present invention a biosensor is provided. The biosensor comprises a support substrate, an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and at least one reagent positioned on at least one electrode.

According to another aspect of the present invention a biosensor is provided. The biosensor comprises a support substrate, an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and a cover cooperating with the support substrate to define a channel. At least a portion of the electrodes are positioned in the channel.

In addition, a method of forming a biosensor is provided in accordance with the present invention. The method comprises the steps of providing a substrate coated with a electrically conductive material, ablating the electrically conductive material to form electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and applying a reagent to at least one of the electrodes.

Still further, in accordance with the present invention a biosensor is provided. The biosensor comprises a support substrate and an electrically conductive coating

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positioned on the support substrate. The coating is formed to define electrodes and means for identifying the biosensor, wherein there is sufficient contrast between the conductive coating and the substrate such that the identifying means is discernible.

Additional features of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the preferred embodiment exemplifying the best mode of carrying out the invention as presently perceived.

BRIEF DESCRIPTION OF THE DRAWINGS

The detailed description particularly refers to the accompanying figures in which:

Fig. 1 is a perspective view of a biosensor in accordance with the present invention, showing the biosensor formed to include a code pattern formed thereon.

Fig. 2 is an exploded assembly view of the biosensor of Fig. 1, showing the biosensor including an electrode array positioned at one end, a spacer substrate including a notch, and a cover formed to extend over a portion of the notch.

Fig. 3 is a view taken along lines 3-3 of Fig. 1.

Fig. 4 is a view taken along lines 4-4 of Fig. 1.

Fig. 5 is an enlarged top view of an alternative code pattern formed on a biosensor in accordance with the present invention.

Fig. 6 is an enlarged top view of an alternative code pattern formed on a biosensor in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a biosensor and a method for manufacturing a biosensor that has a specific code pattern. This code pattern is beneficially formed from the same electrically conductive material and in the same manner as the electrodes of the biosensor, which reduces steps in the manufacturing process. Laser ablation is preferably used in forming the code pattern while generating the electrode pattern. The code pattern can be read in a number of ways, non-limiting examples of which include optically or electrically depending on the structures formed onto the biosensor. The structures could show contrast in their optical reflectivity, their electrical conductivity, or their resistance respectively. The structures could also be high reflectivity areas surrounded by low reflectivity areas or vice versa, or areas of

high electrical conductivity surrounded by areas of low conductivity. Aspects of the invention are presented in Figs. 1-6, which are not drawn to scale and wherein like components in the several views are numbered alike.

Figs. 1-4 illustrate an aspect of the invention in the form of a biosensor 10 having an electrode-support substrate 12, an electrical conductor 13 positioned on the substrate 12 that is disrupted to define electrodes 14, 16, a spacer substrate 18 positioned on substrate 12, and a cover substrate 20 positioned on the spacer substrate 18. Biosensor 10 is preferably rectangular in shape. It is appreciated however, that biosensor 10 can assume any number of shapes in accordance with this disclosure. Biosensor 10 is preferably produced from rolls of material however, it is understood that biosensor 10 can be constructed from individual sheets in accordance with this disclosure. Thus, the selection of materials for the construction of biosensor 10 necessitates the use of materials that are sufficiently flexible for roll processing, but which are still rigid enough to give a useful stiffness to finished biosensor 10.

Referring to Fig. 4, the support substrate 12 includes a first surface 22 facing the spacer substrate 18 and a second surface 24. In addition, as shown in Fig. 2, substrate 12 has opposite first and second ends 26, 28 and opposite edges 30, 32 extending between the first and second ends 26, 28. Substrate 12 is generally rectangular in shape, it is appreciated however, that support may be formed in a variety of shapes and sizes in accordance with this disclosure. Substrate 12 is formed of a flexible polymer and preferably from a flexible polymer and preferably from a polymer such as a polyester or polyimide, polyethylene naphthalate (PEN). A non-limiting example of a suitable PEN is 5 mil (125 um) thick KALADEX®, a PEN film commercially available from E.I. DuPont de Nemours, Wilmington, Delaware, which is coated with gold by ROWO Coating, Henbolzhelm, Germany.

Electrodes 14, 16 are created or isolated from conductor 13 on first surface 22 of substrate 12. Non-limiting examples of a suitable electrical conductor 13 include aluminum, carbon (such as graphite), cobalt, copper, gallium, gold, indium, iridium, iron, lead, magnesium, mercury (as an amalgam), nickel, niobium, osmium, palladium, platinum, rhenium, rhodium, selenium, silicon (such as highly doped polycrystalline silicon), silver, tantalum, tin, titanium, tungsten, uranium, vanadium, zinc, zirconium, mixtures thereof, and alloys, oxides, or metallic compounds of these elements. Preferably, electrical conductor 13 is selected from the following materials: gold, platinum, palladium, iridium, or alloys of these metals, since such noble metals

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and their alloys are unreactive in biological systems. Most preferably, electrical conductor 13 is gold.

Electrodes 14, 16 are isolated from the rest of the electrical conductor 13 by laser ablation. See Fig. 4. Techniques for forming electrodes on a surface using laser ablation are known. See, for example, U.S. Patent Application No. 09/411,940, filed October 4, 1999, and entitled "LASER DEFINED FEATURES FOR PATTERNED LAMINATES AND ELECTRODE", the disclosure of which is expressly incorporated herein by reference. Preferably, electrodes 14, 16 are created by removing the electrical conductor 13 from an area extending around the electrodes to form a gap of exposed support substrate 12. Therefore, electrodes 14, 16 are isolated from the rest of the electrically-conductive material on substrate 12 by a gap having a width of about 25 μm to about 500 μm, preferably the gap has a width of about 100 μm to about 200 μm. Alternatively, it is appreciated that electrodes 14, 16 may be created by laser ablation alone on substrate 12. It is appreciated that while laser ablation is the preferred method for forming electrodes 14, 16 given its precision and sensitivity, other techniques such as lamination, screen-printing, or photolithography may be used in accordance with this disclosure.

As shown in Fig. 2, electrodes 14, 16 cooperate with one another to define an electrode array 36. In addition, electrodes 14, 16 each include a contact 34 and a lead 38 extending between the contact 34 and the array 36. It is appreciated that the leads 38 extending from the array can be formed to have many lengths and extend to a variety of locations on the electrode-support substrate 12. It is appreciated that the configuration of the electrode array, the number of electrodes, as well as the spacing between the electrodes may vary in accordance with this disclosure and that a greater than one array may be formed as will be appreciated by one of skill in the art.

Referring again to Figs. 2 and 3, a recess 35 is formed from the electrical conductor 13 by laser ablation using techniques as described above. Recess is created by removing the electrical conductor 13 to expose the first surface 22 of the support substrate 12 adjacent to the first end 26. It is appreciated that a portion of the first surface 22 may also be removed to form the recess 35 in accordance with this disclosure.

In addition, as shown in Figs. 1, 2, and 4, the discernible code pattern 40 is formed from the electrical conductor 13 by laser ablation using techniques as described above with reference to electrodes 14, 16. Specifically, the code pattern 40

is created by removing the electrical conductor 13 in a pre-defined pattern to expose the first surface 22 of the support substrate 12. While pattern 40 is illustratively a barcode type pattern, it is appreciated that the pattern 40 can take on any number of shapes and patterns, non-limiting examples of which are shown in Figs. 5 and 6.

It is also appreciated that the pattern 40 can be provided in a human readable, optical readable, or electrical readable form in accordance with this disclosure. The structures could show contrast in their optical reflectivity, their electrical conductivity, or their resistivity respectively. To aid in contrasting the electrical conductivity of the code pattern 40, the electrical conductor 13 of the pattern 40 may be coated with a second conductive material (not shown) that is different from the electrical conductor 13. Non-limiting examples of the second conductive material include carbon and silver. It is appreciated, however, that a wide variety of materials may be coated on the electrical conductor 13 to change the electrical property of the code pattern 40.

It is also appreciated; electrodes 14, 16 could be formed from layers of electrically conductive materials having different colors, reflectivity, conductance, etc. Thus, the code pattern can be formed by removing a portion of the electrical conductor layers, leaving behind areas of high reflectivity surrounded by low reflectivity areas or vice versa, areas of high electrical conductivity surrounded by areas of low conductivity or vise versa. It is also possible to laser etch a code pattern that has a known resistance and this area can be read electrochemically to identify or recognize the code pattern. Moreover, it is appreciated that the code pattern can be a combination of any of the above readable forms in accordance with the present invention.

As shown in Fig. 4, the code pattern 40 is isolated from the rest of the electrically conductive material 13 on substrate 12 by gaps 42. Gaps 42 can have a wide variety of widths in accordance with this disclosure depending upon the specific use of the code pattern 40. Non-limiting examples of widths of the gaps include from about 1 μm to about 1000 μm. Alternatively, it is appreciated that the code pattern 40 may be created by laser ablation alone on substrate 12. It is appreciated that while laser ablation is the preferred method for forming the code pattern 40 given its precision and sensitivity, other techniques such as lamination, screen-printing, or photolithography may be used in accordance with this disclosure.

The manufacturer of biosensor 10 may maintain a central database containing a set of code patterns, each of which uniquely identifies an individual biosensor, or

Applicant: Roche Diagnostics Operations, Inc.

batch of biosensors. There may also be associated with each code pattern a set of calibration data for the biosensor 10. It is appreciated that the code patterns may be associated with any number of identification or data sets in accordance with the present invention.

Spacer substrate 18 of biosensor 10 includes an upper surface 44 and a lower surface 46 facing the substrate 12. In addition, the spacer substrate 18 includes opposite first and second ends 48, 50. First end 48 includes a notch 52, which is defined by a border 54. The border illustratively includes three generally linear sides. It is appreciated that the notch can take on a variety of shapes and sizes in accordance with this disclosure. When biosensor 10 is assembled, the border 54 extends about at least a portion of the array 36 so that the array 36 is at least partially exposed in the notch 52.

Spacer substrate 18 is formed of a flexible polymer and preferably from a flexible polymer and preferably from a polymer such as an adhesive coated polyethylene terephthalate (PET) polyester. A non-limiting example of a suitable PET is 3 mil (75 um) thick white PET film both sides of which are coated with a pressure-sensitive adhesive (Product # ARcare 8877) commercially available from Adhesives Research, Inc. Glen Rock, Pennsylvania. It is appreciated that spacer substrate 18 may be constructed of a variety of materials and may be coupled to the substrate 12 and the cover substrate 20 using a wide variety of commercially available adhesives, or by welding (heat or ultrasonic) when large portions of the surface 22 of the electrode support substrate 12 are exposed and not covered by electrical conductor 13.

The cover substrate 20 is coupled to the upper surface 44 of the spacer substrate 18. See Fig. 3. The cover substrate 20 includes opposite first and second ends 56, 58. The cover substrate 20 is coupled to the spacer substrate 18 such that the first end 56 is spaced-apart from the end 48 of the spacer substrate 18 and the second end 58 is spaced-apart from the end 50 of the spacer substrate 18. When biosensor 10 is assembled, cover substrate 20 cooperates with the spacer support 20 and the electrode-support 12 to define a capillary channel 60.

Cover substrate 20 is generally rectangular in shape, it is appreciated, however, that the cover substrate may be formed in a variety of shapes and sizes in accordance with this disclosure. Cover substrate 20 is formed from a flexible polymer and preferably from a polymer such as polyester. A non-limiting example of a

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suitable polymer is 3.9 mil (99 um) thick 3M hydrophilic polyester film (3M Product #9971), commercially available from 3M Healthcare, St. Paul, MN.

Referring now to Figs. 1 and 3, the capillary channel 60 is generally linear in shape and is defined by the cover substrate 20, the electrode support substrate 12, and the border 54 of the spacer substrate 18. When biosensor 10 is assembled, channel 60 extends across the electrode array 36. Cover substrate 20 does not extend across the entire notch 52, therefore, a portion of the notch serves as an air outlet in accordance with this disclosure.

An electrochemical reagent 62 is positioned on the array 36. The reagent 62 provides electrochemical probes for specific analytes. The term analyte, as used herein, refers to the molecule or compound to be quantitatively determined. Non-limiting examples of analytes include carbohydrates, proteins, such as hormones and other secreted proteins, enzymes, and cell surface proteins; glycoproteins; peptides; small molecules; polysaccharides; antibodies (including monoclonal or polyclonal Ab); nucleic acids; drugs; toxins; viruses of virus particles; portions of a cell wall; and other compounds processing epitopes. The analyte of interest is preferably glucose.

The choice of the specific reagent 62 depends on the specific analyte or analytes to be measured, and are well known to those of ordinary skill in the art. An example of a reagent that may be used in biosensor 10 of the present invention is a reagent for measuring glucose from a whole blood sample. A non-limiting example of a reagent for measurement of glucose in a human blood sample contains 62.2 mg polyethylene oxide (mean molecular weight of 100-900 kilo Daltons), 3.3 mg NATROSOL 244M, 41.5 mg AVICEL RC-591 F, 89.4 mg monobasic potassium phosphate, 157.9 mg dibasic potassium phosphate, 437.3 mg potassium ferricyanide, 46.0 mg sodium succinate, 148.0 mg trehalose, 2.6 mg TRITON X-100 surfactant, and 2,000 to 9,000 units of enzyme activity per gram of reagent. The enzyme is prepared as an enzyme solution from 12.5 mg coenzyme PQQ and 1.21 million units of the apoenzyme of quinoprotein glucose dehydrogenase. This reagent is further described in U.S. Patent No. 5,997,817, the disclosure of which is expressly incorporated herein by reference.

Non-limiting examples of enzymes and mediators that may be used in measuring particular analytes in biosensor 10 are listed below in Table 1.

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TABLE 1

Analyte	Enzymes	Mediator (Oxidized Form)	Additional Mediator
Glucose	Glucose Dehydrogenase and Diaphorase	Ferricyanide	
Glucose	Glucose-Dehydrogenase (Quinoprotein)	Ferricyanide	
Cholesterol	Cholesterol Esterase and Cholesterol Oxidase	Ferricyanide	2,6-Dimethyl-1,4-Benzoquinone 2,5-Dichloro-1,4-Benzoquinone or Phenazine Ethosulfate
HDL Cholesterol	Cholesterol Esterase and Cholesterol Oxidase	Ferricyanide	2,6-Dimethyl-1,4-Benzoquinone 2,5-Dichloro-1,4-Benzoquinone or Phenazine Ethosulfate
Triglycerides	Lipoprotein Lipase, Glycerol Kinase, and Glycerol-3-Phosphate Oxidase	Ferricyanide or Phenazine Ethosulfate	Phenazine Methosulfate
Lactate	Lactate Oxidase	Ferricyanide	2,6-Dichloro-1,4-Benzoquinone
Lactate	Lactate Dehydrogenase and Diaphorase	Ferricyanide Phenazine Ethosulfate, or Phenazine Methosulfate	
Lactate Dehydrogenase	Diaphorase	Ferricyanide	Phenazine Ethosulfate, or Phenazine Methosulfate
Pyruvate	Pyruvate Oxidase	Ferricyanide	
Alcohol	Alcohol Oxidase	Phenylenediamine	
Bilirubin	Bilirubin Oxidase	1-Methoxy-Phenazine Methosulfate	
Uric Acid	Uricase	Ferricyanide	

In some of the examples shown in Table 1, at least one additional enzyme is used as a reaction catalyst. Also, some of the examples shown in Table 1 may utilize an additional mediator, which facilitates electron transfer to the oxidized form of the mediator. The additional mediator may be provided to the reagent in lesser amount than the oxidized form of the mediator. While the above assays are described, it is contemplated that current, charge, impedance, conductance, potential, or other electrochemically indicated property of the sample might be accurately correlated to

the concentration of the analyte in the sample with biosensor 10 in accordance with this disclosure.

A plurality of biosensors 10 are typically packaged in a vial, usually with a stopper formed to seal the vial. It is appreciated, however, that biosensors 10 may be packaged individually, or biosensors can be folded upon one another, rolled in a coil, stacked in a cassette magazine, or packed in blister packaging.

Biosensor 10 is used in conjunction with the following:

1. a power source in electrical connection with contacts 34 and capable of supplying an electrical potential difference between electrodes 14, 16 sufficient to cause diffusion limited electro-oxidation of the reduced form of the mediator at the surface of the working electrode; and

2. a meter in electrical connection with contacts 34 and capable of measuring the diffusion limited current produced by oxidation of the reduced form of the mediator with the above-stated electrical potential difference is applied.

The meter is provided with a pattern reader that is capable of reading the code pattern 40 into a memory of the meter. The reader can be an electrical or optical reader in accordance with the present invention. The reader is formed to read the code pattern 40 when the biosensor 10 is inserted into the meter. When, however, the code pattern is in a human readable form, it is appreciated that the meter may include an interface, which permits the user to input the information from the code pattern manually. There are many ways to optically read code pattern 40 such as laser scanners, pen-like wands, and charge-couple-device (CCD) scanners. A non-limiting example of a suitable optical reader suitable for use with the present invention includes a light emitting diode(s) (LED), a lens, and a photodiode. It is appreciated that the reader may be an independent internal component of the meter.

The meter may further be formed to transfer the code pattern from the meter to a memory unit where it is stored. It is appreciated that the memory unit can be formed to store information regarding the specifics of the code pattern as well as patient information including previous meter readings. The meter will normally be adapted to apply an algorithm to the current measurement, whereby an analyte concentration is provided and visually displayed. Improvements in such power source, meter, and biosensor system are the subject of commonly assigned U.S. Pat. No. 4,963,814, issued Oct. 16, 1990; U.S. Pat. No. 4,999,632, issued Mar. 12, 1991; U.S. Pat. No. 4,999,582, issued Mar. 12, 1991; U.S. Pat. No. 5,243,516, issued Sep. 7,

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1993; U.S. Pat. No. 5,352,351, issued Oct. 4, 1994; U.S. Pat. No. 5,366,609, issued Nov. 22, 1994; White et al., U.S. Pat. No. 5,405,511, issued Apr. 11, 1995; and White et al., U.S. Pat. No. 5,438,271, issued Aug. 1, 1995, the disclosures of each of which are expressly hereby incorporated by reference.

Many fluid samples may be analyzed. For example, human body fluids such as whole blood, plasma, sera, lymph, bile, urine, semen, cerebrospinal fluid, spinal fluid, lacrimal fluid and stool specimens as well as other biological fluids readily apparent to one skilled in the art may be measured. Fluid preparations of tissues can also be assayed, along with foods, fermentation products and environmental substances, which potentially contain environmental contaminants. Preferably, whole blood is assayed with this invention.

To manufacture biosensor 10 a roll of metallized electrode support material is fed through guide rolls into an ablation/washing and drying station. A laser system capable of ablating support 12 is known to those of ordinary skill in the art. Non-limiting examples of which include excimer lasers, with the pattern of ablation controlled by mirrors, lenses, and masks. A non-limiting example of such a custom fit system is the LPX-300 or LPX-200 both commercially available from LPKF Laser Electronic GmbH, of Garbsen, Germany.

In the laser ablation station, the metallic layer of the metallized film is ablated in a pre-determined pattern, to form a ribbon of isolated electrode sets on the electrode support material, code patterns, and a recess in the film adjacent to each electrode array. To ablate electrodes 14, 16, recess 35, and code patterns 40 in 50 nm thick gold conductor 13, 90 mJ/cm^2 energy is applied. It is appreciated, however, that the amount of energy required may vary from material to material, metal to metal, or thickness to thickness. The ribbon is then passed through more guide rolls, with a tension loop and through an optional inspection system where both optical and electrical inspection can be made. The system is used for quality control in order to check for defects.

Upon leaving the laser ablation station, the metallized film is fed into a reagent dispensing station. Reagents that have been compounded are fed into a dispensing station where it is applied in a liquid form to the center of respective the array 34. Reagent application techniques are well known to one of ordinary skill in the art as described in U.S. Patent No. 5,762,770, the disclosure of which is expressly incorporated herein by reference. It is appreciated that reagents may be applied to the

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array 34 in a liquid or other form and dried or semi-dried onto the array 34 in accordance with this disclosure.

In a separate process, a double-sided pressure-sensitive film with dual release liners is fed into a window punch unit where notches are formed. The film is then fed into a lamination & kiss-cutting station. At the same time, a roll of cover substrate material is fed over a guide roll into the lamination & kiss-cutting station, where the release liner is removed from the upper surface 44 and rewound into a roll. The upper surface 33 of the spacer substrate material is applied to the cover substrate material. Next, the film is kiss cut and a portion of the cover substrate material is removed, leaving behind the cover substrate material coupled to the spacer substrate material, extending across a portion of the notch.

The cover material/spacer substrate subassembly is fed into a sensor lamination & cut/pack station. The reagent-coated electrode-support substrate material is fed from the dispensing station into the sensor lamination & cut/pack station as well. The remaining release liner is removed from the spacer substrate and the spacer substrate is positioned on the electrode-support substrate material so that at least a portion of the electrode array 36 is aligned with the notch 52. Next, the resulting assembled material is cut to form individual biosensors 10, which are sorted and packed into vials, each closed with a stopper, to give packaged biosensor strips.

In use, the meter is turned on and the biosensor is inserted into the meter. It is appreciated that the user may turn on the meter, or it may turn on automatically upon insertion of the biosensor. The LED emits a light that is directed through a lens towards the code pattern of the biosensor. The light is reflected off of the code pattern, through the lens, and toward the photodiode. The photodiode measures the intensity of the light that is reflected back from the code pattern and generates a corresponding voltage waveform. A decoder deciphers this waveform and translates it into a reading of the code pattern. It is appreciated that many commercially available optical readers may be used in accordance with the present invention. Preferably, the optical reader will be custom fit reader.

In use, a user of biosensor 10 places a finger having a blood collection incision against the recess 35 in the notch 52. Capillary forces pull a liquid blood sample flowing from the incision through the capillary channel 60 across the reagent 62 and the array 34. The liquid blood sample dissolves the reagent 62 and engages the array 34 where the electrochemical reaction takes place.

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In use for example, after the reaction is complete, a power source (e.g., a battery) applies a potential difference between the electrodes 14, 16 respectively. When the potential difference is applied, the amount of oxidized form of the mediator at the reference electrode and the potential difference must be sufficient to cause diffusion-limited electro-oxidation of the reduced form of the mediator at the surface of the working electrode. A current measuring meter (not shown) measures the diffusion-limited current generated by the oxidation of the reduced form of the mediator at the surface of the working electrode.

The measured current may be accurately correlated to the concentration of the analyte in sample when the following requirements are satisfied:

1. The rate of oxidation of the reduced form of the mediator is governed by the rate of diffusion of the reduced form of the mediator to the surface of the working electrode.

2. The current produced is limited by the oxidation of reduced form of the mediator at the surface of the working electrode.

The processes and products described above include disposable biosensor 10 especially for use in diagnostic devices. Also included, however, are electrochemical sensors for non-diagnostic uses, such as measuring an analyte in any biological, environmental, or other sample. As discussed above, biosensor 10 can be manufactured in a variety of shapes and sizes and be used to perform a variety of assays, non-limiting examples of which include current, charge, impedance conductance, potential or other electrochemical indicative property of the sample applied to biosensor.

In accordance with another embodiment of the present invention, biosensor 110 is illustrated in Fig. 5. Biosensor 110 is formed in a similar manner to biosensor 10 except that biosensor 110 includes a code pattern 140. Code pattern 140 includes nine isolated pads 160. It is appreciated that the number of pads can be greater or fewer than nine in accordance with this disclosure. Each pad 160 is separated by from the surrounding electrical conductor by a gap 170.

Code pattern 140 is used once biosensor 110 is attached to a meter circuit board (not shown) that includes a connector. Generally, the connector will include two contacts per possible pad location on biosensor 110. Code pattern 140 of the present invention enables the meter to check continuity at each pad 160 location or determine that a pad does not exist in a pre-determined location. If a pad 160 is

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present, the meter will recognize the presence of a pad 160 by a continuity check. One of ordinary skill in the art will be well aware of methods suitable for performing a continuity check.

Code pattern 140 is formed from the electrical conductor by laser ablation using techniques as described above with reference to electrodes 14, 16, shown for example in Fig. 1. Specifically, removing the electrical conductor in a pre-defined pattern to expose the first surface of the support substrate 12 creates the code pattern 140. Code pattern 140 can also be coated with a second electrical conductor (not shown) to modify the electrical resistivity of the pattern 140. While pattern 140 illustratively includes nine spaced-apart generally square-shaped pads, it is appreciated that the pattern 140 can take on any number of shapes and patterns in accordance with this disclosure. In addition, it is appreciated that the pattern 140 can be read optically or electrically in accordance with this disclosure.

In use, when the user inserts biosensor 110 into the meter (not shown), the biosensor 110 makes contact to the connector and the electronics of the meter inquire as to how many pads 160 are showing continuity. Predetermined lot information may be stored in a memory unit of the meter. It is appreciated that the memory unit may also store a variety of patient information including previous meter readings. This memory unit is formed with memory components, a non-limiting example of which is known as RAM, which is well known in the prior art. The results of the continuity query may be used to set the appropriate code information in the meter, which enables the meter to eliminate chemistry or reagent variation.

In accordance with another embodiment of the present invention, biosensor 210 is illustrated in Fig. 6. Biosensor 210 is formed in a similar manner to biosensor 10, except that biosensor 210 includes a code pattern 240. Code pattern 240 includes nine pads 260 that are in communication with one another. It is appreciated that the number of pads can vary in accordance with this disclosure. Each pad 260 is separated from the surrounding electrical conductor by gaps 270.

Code pattern 240 is formed from the electrical conductor by laser ablation using techniques as described above with reference to electrodes 14, 16, shown for example in Fig. 1. Specifically, removing the electrical conductor in a pre-defined pattern to expose the first surface of the support substrate 12 creates the code pattern 240. Code pattern 240 can also be coated with a second electrical conductor (not shown) to modify the electrical resistivity of the pattern 240.

While pattern 240 illustratively includes nine generally square-shaped pads that are interconnected, it is appreciated that the pattern 240 can take on any number of shapes and patterns in accordance with this disclosure, which would give various resistance levels. These differing resistance levels can be correlated to a reagent lot. For example, the pattern 240 can be varied by disconnecting the internal links between the pads 260. This disconnection can be done, for example, by a laser. By changing the number of interconnected pads, the resistance of the remaining interconnected pads 260 will be different. In addition, it is appreciated that the pattern 240 can be read optically or electrically in accordance with this disclosure.

In use, when the user inserts biosensor 210 into the meter (not shown), the biosensor 210 makes contact to the connector and the electronics of the meter inquire as to how many pads 260 are showing continuity. Information related to this continuity is similar to that previously described with reference to biosensor 110.

In addition, the biosensor 210 will make contact with electronics of the meter, which determines the resistance between the interconnected pads. Thus, in preferred embodiments, the meter will determine which pads exist on the biosensor 210, and the resistance of the interconnected pads 260. The information can be stored in the meter as described above with reference to biosensors 10 and 110.

Although the invention has been described in detail with reference to a preferred embodiment, variations and modifications exist within the scope and spirit of the invention, on as described and defined in the following claims.

Doc#: 2045-0015.01

What is claimed is:

1. A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and

at least one reagent positioned on at least one electrode.

2. The biosensor of claim 1 wherein the code pattern is a bar code.

3. The biosensor of claim 1 wherein the conductive coating is gold.

4. The biosensor of claim 1 wherein the code pattern is optically discernible.

5. The biosensor of claim 1 further comprising a second electrically conducive coating positioned on the code pattern.

6. The biosensor of claim 1 wherein the code pattern is electrically discernible.

7. The biosensor of claim 1 wherein the code pattern includes recesses formed in the conductive coating.

8. The biosensor of claim 1 wherein the support substrate includes opposite first and second ends and the code pattern is positioned adjacent to the second end.

9. The biosensor of claim 1 further comprising a cover substrate extending across at least a portion of the electrodes.

10. The biosensor of claim 1 wherein code pattern includes pads that are spaced-apart from the surrounding electrically conductive coating.

11. The biosensor of claim 10 wherein the pads are isolated from one another.

12. The biosensor of claim 10 wherein the pads are interconnected.

13. A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and

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a cover cooperating with the support substrate to define a channel and at least a portion of the electrodes are positioned in the channel.

14. The biosensor of claim 13 wherein the code pattern is optically discernible.

15. The biosensor of claim 13 further comprising a second electrically conducive coating positioned on the code pattern.

16. The biosensor of claim 13 wherein the code pattern is electrically discernible.

17. The biosensor of claim 13 wherein the support substrate includes opposite first and second ends and the code pattern is positioned adjacent to the second end.

18. The biosensor of claim 17 wherein the electrodes cooperate to define an array positioned adjacent to the first end.

19. The biosensor of claim 18 wherein the channel extends from the first end to the array.

20. The biosensor of claim 13 wherein the code pattern is a bar code.

21. The biosensor of claim 20 wherein the conductive coating is gold.

22. The biosensor of claim 13 wherein code pattern includes pads that are spaced-apart from the surrounding electrically conductive coating.

23. The biosensor of claim 22 wherein the pads are isolated from one another.

24. The biosensor of claim 22 wherein the pads are interconnected.

25. A method of forming a biosensor, the method comprising the steps of:

providing a substrate coated with a electrically conductive material,

ablating the electrically conductive material to form electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and

applying a reagent to at least one of the electrodes.

26. The method of claim 25 wherein the ablating step includes the step of laser ablating the electrically conductive material.

27. The method of claim 26 wherein the ablating step includes forming a bar code shaped code pattern.

Doc#: 6616616 - 06/09/2003

28. The method of claim 25 wherein the ablating step includes forming a code pattern that includes pads isolated from the surrounding electrically conductive material.

29. The method of claim 28 wherein the pads are interconnected.

30. A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and means for identifying the biosensor, wherein there is sufficient contrast between the conductive coating and the substrate such that the identifying means is discernible.

31. The biosensor of claim 30 further comprising a cover that cooperates with the support substrate to define a channel and at least a portion of the electrodes are positioned in the channel.

32. The biosensor of claim 30 wherein the identifying means is a bar code.

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ABSTRACT OF THE DISCLOSURE

The present invention relates to a biosensor. The biosensor includes a support substrate, an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and a cover cooperating with the support substrate to define a channel. At least a portion of the electrodes are positioned in the channel.

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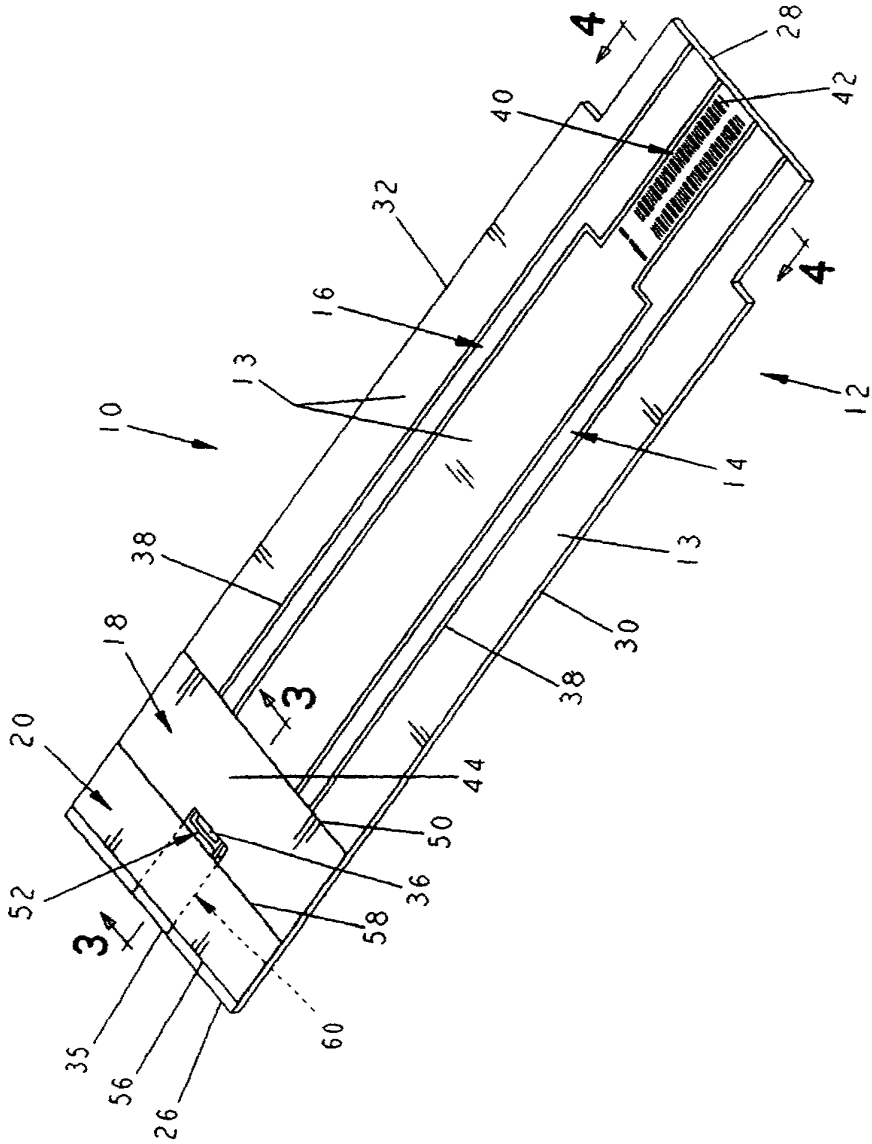


FIG. 1

09945515 "082904

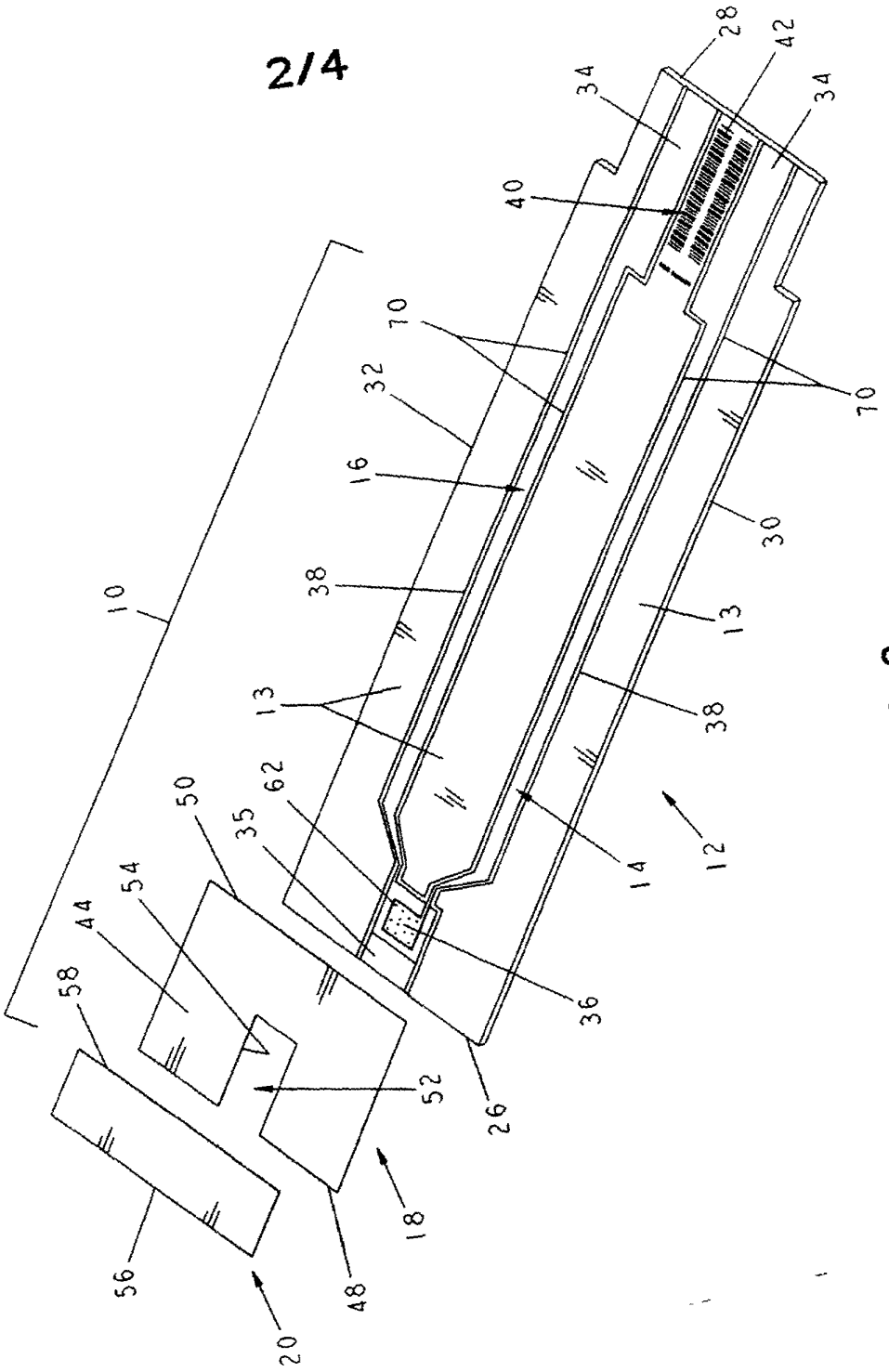


FIG. 2

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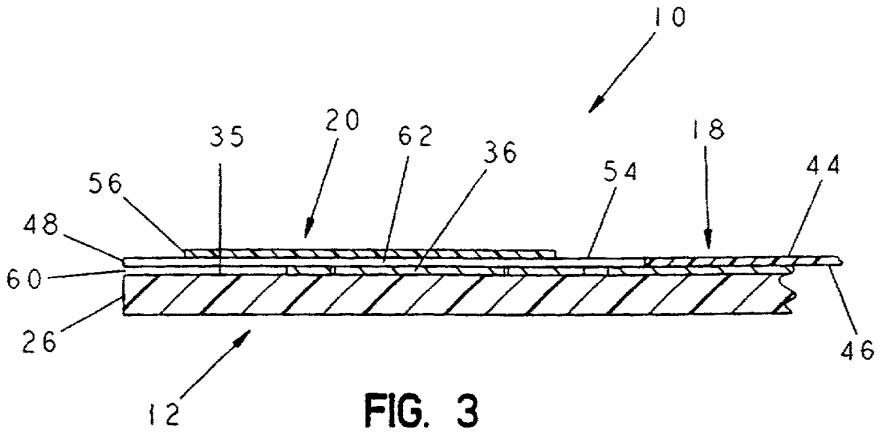


FIG. 3

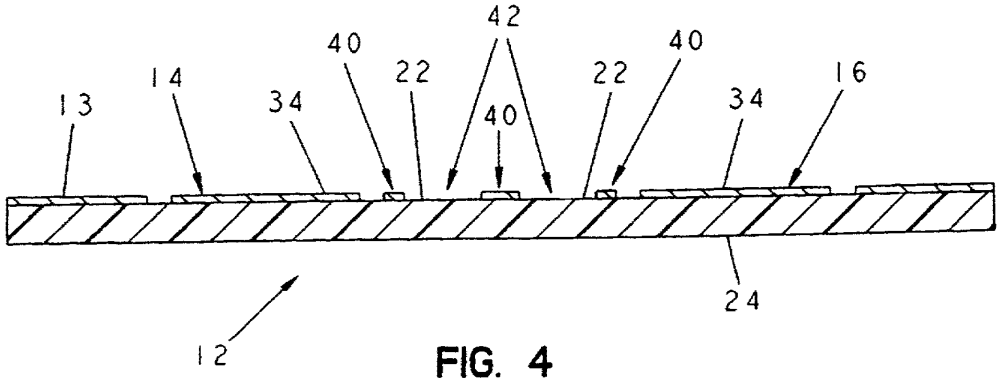


FIG. 4

09045455 "090290

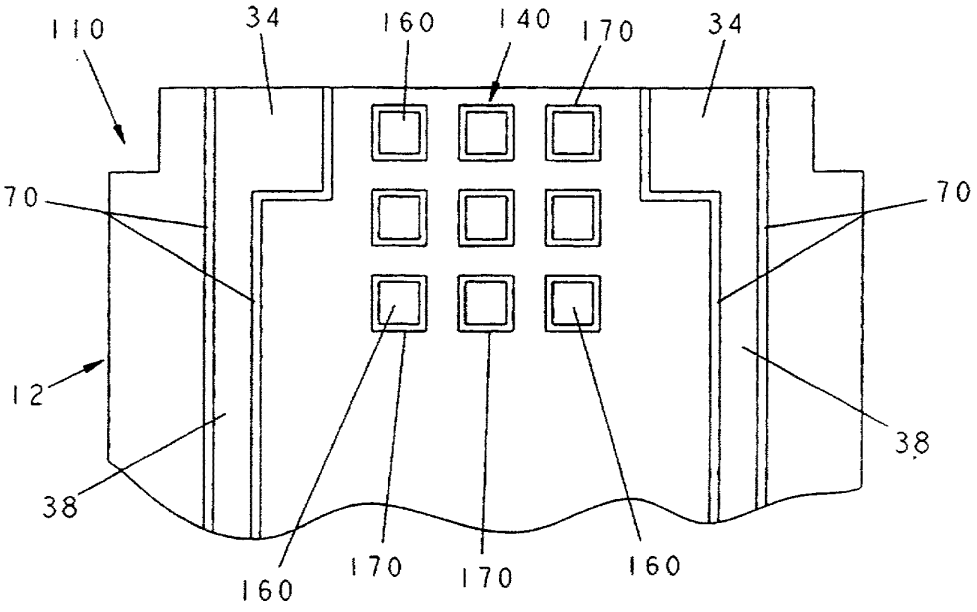


FIG. 5

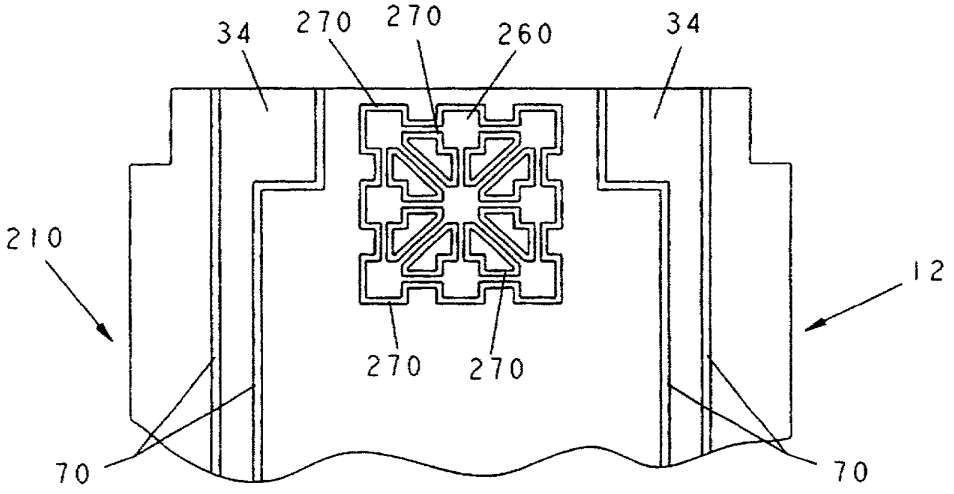


FIG. 6

09425365.062201

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

BIOSENSOR

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International Application Number _____

and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

BIOSENSOR

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International Application Number _____

☐ and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined, in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or

agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Jill Lynn Woodburn, Reg. No, 39,874
D. Michael Young, Reg. No. 33,819
Brent A. Harris, Reg. No. 39,215
Richard T. Knauer, Reg. No. 35,575
Kenneth J. Waite, Reg. No. 45,189
Marilyn L. Amick, Reg. No. 31,444
Michelle Neff, Reg. No. 47,817

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The Law Office of Jill L. Woodburn, L.L.C.
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Direct Telephone Calls to: *(name and telephone number)*

Jill Lynn Woodburn, Telephone No. (812) 842-2660

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Second inventor's signature Date
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Sixth inventor's signature James L Pauley Jr Date 8/24/01
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Citizenship US
Post Office Address 1710 Ruddy Lane
Fishers, IN 46038

CLAIMS ONLY

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND	DEP	IND	DEP	IND	DEP
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TOTAL DEP						
TOTAL CLAIMS						

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 2000

Application or Docket Number

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS		
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	minus 20=	*
INDEPENDENT CLAIMS	minus 3 =	*
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	355.00	OR	BASIC FEE	710.00
X$ 9=		OR	X$18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL		OR	TOTAL	

CLAIMS AS AMENDED - PART II

AMENDMENT A	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL ADDT FEE		OR	TOTAL ADDT FEE	

AMENDMENT B	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL ADDT FEE		OR	TOTAL ADDT FEE	

AMENDMENT C	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	
Independent	*	Minus	***	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL ADDT FEE		OR	TOTAL ADDT FEE	

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

09/04/2001 BSAYASI1 00000009 022958 09942515

01 FC:101 710.00 CH
02 FC:102 80.00 CH
03 FC:103 216.00 CH

PTO-1556
(5/87)

*U.S. GPO: 1999-459-082/19144

JC828 U.S. PTO
09/942515
08/29/01

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group: Unknown

Attorney Docket: RDID 00108 US

Applicant: Bhullar et al.

Invention: Biosensor

Serial No.: To be Assigned

Filed: Herewith (Aug. 29, 2001)

Examiner: Unknown

INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to 37 CFR 1.98, the following information is brought to the attention of the U.S. Patent Office.

It is not believed that the disclosure contained in any of the references listed on the attached Form 1449 anticipates Applicants' invention, or that any of the disclosures, alone, or in combination with other disclosures, render Applicants' invention obvious. The Examiner, however, is invited to read each reference in its entirety to ascertain its relevance to the present application and claimed invention. The Examiner is also asked to make each reference of record.

Respectfully submitted,

Date: August 29, 2001

Jill L. Woodburn
Reg. 39,874
Attorney for Applicant

The Law Office of Jill L. Woodburn, L.L.C.
6633 Old Stonehouse Dr.
Newburgh, IN 47630
812/842-2660

U.S. Department of Commerce Patent and Trademark Office	ATTY DOCKET NO. RDID 00108	SERIAL NO. To Be Assigned
	APPLICANT Bhullar et al.	
INFORMATION DISCLOSURE STATEMENT	FILING DATE Herewith	GROUP To Be Assigned

JC828 U.S. PTO
09/942515
08/29/01

*Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
	AA	4,476,149	10/9/84	Poppe et al.	427	2	
	AB	4,592,893	6/3/86	Poppe et al.	422	56	
	AC	4,671,661	6/9/87	Ott	356	402	
	AD	4,963,814	10/16/90	Parks et al.	323	274	
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Examiner	Date Considered

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U.S. Department of Commerce Patent and Trademark Office	ATTY DOCKET NO. RDID 0003 US	SERIAL NO. To Be Assigned
	APPLICANT Bhullar et al.	
INFORMATION DISCLOSURE STATEMENT	FILING DATE Herewith	GROUP To Be Assigned

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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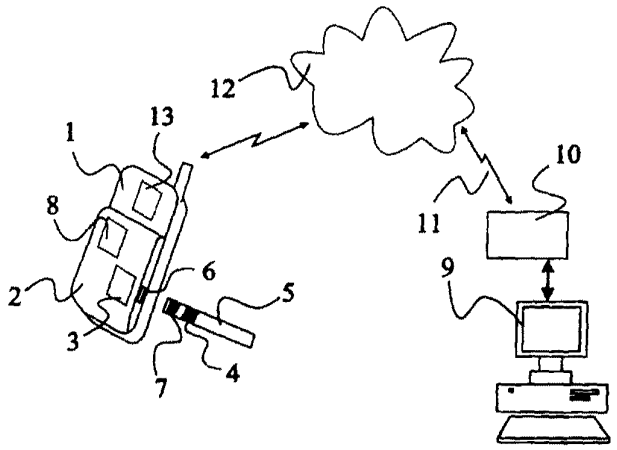
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(54) Title: CALIBRATION OF MEASURED PHYSICAL PARAMETERS



(57) Abstract

A system for measuring the blood glucose level in a sample of a patient's blood. Consumable test strips (5) are provided together with a code (7) which identifies the manufacturing batch of the strip (5). A measurement unit (2) is provided and is coupled to a mobile telephone (1). The measurement unit (2) is arranged to receive a test strip (5) and to determine a colour change in a reagent (4) due to reaction of the reagent (4) with a blood sample. The identification code (7) is read at the same time by the measurement unit (3) and is transmitted by the mobile telephone (1) to a central database (9) provided by the test strip manufacturer. The database (9) contains identification codes (7) together with associated calibration data. Upon receipt of an identification code (7) the remote database (9) transmits the associated calibration data to the mobile telephone (1) which uses the calibration data to calculate a test result from the measured change in colour. The result can be displayed to the patient on a display of the telephone (1).

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CALIBRATION OF MEASURED PHYSICAL PARAMETERS

The present invention relates to measurement apparatus and in particular to measurement apparatus which makes use of a consumable reagent in a measuring process.

There are many examples of measurement apparatus which make use of a consumable reagent as part of a measuring process. Consumable reagents are generally of a chemical, biochemical, or biological nature. One example of such an apparatus is a blood glucose meter designed to measure the level of glucose in a sample of a patient's blood. A small amount of a suitable reagent is printed or otherwise deposited onto an elongate plastic strip which can be inserted into the blood glucose meter. The meter comprises a reflectometry based measuring system which detects a change in the colour of the printed reagent due to a reaction between the active reagent and glucose present in the blood sample.

It will be appreciated that in the case of a blood glucose meter, where a patient determines an insulin treatment regime on the basis of blood glucose measurements, the accuracy of the meter is critical. This requires very precise calibration of the meter. Initial calibration of the meter is normally carried out during and immediately following manufacturing, with certain calibration data being stored in permanent memory of the meter. However, calibration of the meter at this stage cannot easily account for changes and variations in the properties of the consumable reagents themselves, variations which might arise due to slight changes in the manufacturing process of the reagent and the test strip, environmental factors such a temperature and humidity, and changes in the property of the reagent over time.

It is known to provide blood glucose test strips, or rather a pack of such test strips, with a machine readable memory in which calibration data is stored. When a new pack of test strips is opened, the machine readable memory is read by the meter and calibration data downloaded into the memory of the blood glucose meter. In this way it is possible to take account of reagent manufacturing variations in the calibration of the meter. However, it will be appreciated that providing a machine readable memory with each packet of test strips is relatively expensive both in terms of the hardware required and in terms of the additional time required to program the memory during the manufacturing process.

In addition, it remains difficult to account for changes in the properties of a reagent over time. At best, only a poor estimate can be made based upon some predetermined criteria and only then if the age of the reagent is known. As such, manufacturers of blood glucose test strips tend to supply test strips in very limited numbers and make tight specifications for storage and lifetime to ensure that the properties of the reagents remain within desired limits. These specifications tend to work against economies of scale in the manufacturing and distribution of test strips.

These problems are not limited to blood glucose test strips and apply equally to other fields in which consumable reagents are used.

According to a first aspect of the present invention there is provided a measurement system comprising:

measurement apparatus for measuring a physical parameter using a consumable reagent, said reagent having an identification code associated therewith;

automatic telephone answering means;

a database coupled to the automatic telephone answering means and containing a set of consumable reagent identification codes and respective calibration data;

means for coupling the measurement apparatus to the automatic telephone answering means via a telephone link,

the automatic telephone answering means being arranged to receive an identification code transmitted from the measurement apparatus over said telephone link, to subsequently access said database to identify the calibration data associated with said transmitted identification code, and to either transmit the identified calibration data to the measurement apparatus, or to calculate a test result using the identified calibration data and transmit the test result to the measurement apparatus.

By storing the calibration data for specific consumable reagents at a central database, it is possible to update the calibration data to reflect changes which may occur to the consumable reagents over time. Furthermore, it is no longer necessary to supply the calibration data to the end user with the reagents. This simplifies the manufacturing process and also eliminates the need to supply complex memory means to the end user. A further advantage of the present invention is that data generated by the measurement apparatus using the consumable reagent can be transmitted to the central database where information from many apparatus can be collected and analysed.

In one embodiment of the present invention, the automatic telephone answering means is arranged to transmit calibration data associated with the received identification code back to the measurement apparatus. The measurement apparatus comprises data processing means for processing said measured physical parameter, using the received calibration data, to provide a test result. Preferably, the measurement apparatus comprises a display for displaying the test result to a user.

In an alternative embodiment of the present invention, the measurement apparatus is arranged to transmit said measured physical parameter to the automatic telephone answering means together with said identification code. The system further comprises data processing means, coupled to the database and the automatic telephone answering means, for calculating a test result using the identified calibration data and the received physical parameter. The automatic telephone answering means is arranged to transmit the test result back to the measurement apparatus, e.g. for display on a display of the apparatus. An advantage of this embodiment is that it is possible to use relatively complex algorithms to compute the test result due to the relatively high processing capability which can be provided at a central facility. Furthermore, the algorithm can be easily updated or modified.

Preferably, the identification code is provided with the consumable reagent in a machine readable form. For example, the code may be contained in a bar-code or in a solid state memory. The measurement apparatus is provided with a machine reader which is capable of reading the identification code into a memory of the measurement apparatus. An identification code may be provided with each individual consumable reagent or may be provided with a batch of such units. Where the consumable reagents are provided on a test strip, the code may be provided on one such test strip or on a dummy strip.

In an alternative embodiment, the identification code is provided in a human readable form. The measurement apparatus comprises a user interface for allowing the user to enter the identification code. For example, the user interface may be a keypad.

The measurement apparatus may comprise mobile telephone apparatus which is able to connect the measurement apparatus to the database via a wireless transmission channel. A measurement unit of the apparatus may be integral with said mobile phone or may be connectable thereto. Data may be transmitted between the mobile telephone apparatus and the automatic telephone answering means via a data call. In the case that the mobile

telephone apparatus is a digital telephone apparatus according to the GSM system, the data may be transmitted using the short messaging system (SMS).

The present invention is applicable, for example, to a system for measuring the glucose level in a sample of a patient's blood.

According to a second aspect of the present invention there is provided a consumable reagent, or a pack of consumable reagents, in combination with an identification code, said reagent(s) being for use with the measurement system of the above first aspect of the present invention, where said identification code identifies a set of calibration data stored in said database.

Preferably, the combination of the second aspect of the present invention comprises a machine readable memory by means of which said identification code is stored. The memory may be a bar code, memory chip, magnetic strip, or any other suitable means.

According to a third aspect of the present invention there is provided a method of deriving a test result from a physical parameter measured using a measurement apparatus and a consumable reagent, the method comprising;

providing a consumable reagent together with an identification code;

providing a database containing a set of identification codes and respective calibration data;

transmitting said identification code from the measurement apparatus to the database via a telephone link; and

automatically responding to receipt of the transmitted identification code by identifying the corresponding calibration data in the database, and transmitting that data, or a test result determined using the data, back to the measurement apparatus.

Preferably, said telephone link comprises a mobile telephone link, wherein the measurement apparatus comprises a measurement unit coupled to mobile telephone apparatus.

For a better understanding of the present invention and in order to show how the same may be carried into effect reference will now be made, by way of example, to the accompanying drawings, in which:

Figure 1 shows a measurement system embodying the present invention; and

Figure 2 is a flow diagram illustrating a measurement method implemented by the system of Figure 1.

There is shown in Figure 1 a system which is capable of providing a diabetic patient with a blood glucose level test result. The system comprises a mobile telephone 1 which is shown from the rear in Figure 1 and in which the keyboard and display are not visible. The telephone comprises modified software but is otherwise conventional. However, in place of the conventional battery, a measurement unit 2 is provided which can be inserted into the slot on the rear of the mobile phone 1 normally occupied by the battery. The measurement unit 2 contains a reduced size battery unit (not shown) for powering the measurement unit 2 and the phone 1.

The measurement unit 2 contains an internally arranged reflectometry system 3 which is operable to measure the colour change in a consumable reagent 4 relative to some predetermined base level. The consumable reagent 4 is printed, or otherwise deposited, on a surface region of an elongate test strip 5. The test strip 5 is insertable into a slot 6 provided in the side of the measurement unit 2 and which provides access to the reflectometry system 3.

In addition to the consumable reagent 4, the test strip 5 is provided with a barcode 7 printed on its upper surface. The barcode 7 represents an identification code which identifies the manufacturing batch number of the test strip 5. The measurement unit 2 contains an optical reader, aligned with the slot 6, which reads the barcode 7 when the test strip 5 is inserted into the slot 6. The optical reader may be an independent internal component of the measurement unit 2, but is preferably provided, at least in part, by the reflectometry system 3. In either case, the read identification code is stored in a memory 8 of the measurement unit 2 or of the telephone 1.

The manufacturer or distributor of the test strips 5 maintains a central database 9 containing a set of identification codes, each of which uniquely identifies an individual manufacturing batch of test strips 5. Associated with each identification code is a set of calibration data. This calibration data typically includes an offset value and a scaling factor which can be used to relate a measured colour change to a blood glucose level (non-linear calibration data may also be provided). Calibration data for a batch of test strips is initially determined following manufacture of a batch, for example by comparing the results of a test carried out using a typical measurement unit and by laboratory

analysis. The calibration data however is updated at regular time intervals by re-testing samples taken from a retained test strip stock.

An automatic telephone answering device 10 is located at the manufacturer's (or distributor's) premises and is coupled to both a telephone line 11 and to the database 9. The operation of this device 10 is described below.

To initiate a blood glucose test, the patient activates a measurement sequence using a menu displayed on the phone's display. The patient then deposits a small amount of blood on top of the reagent 4. After a short time, the test strip 5 is fully inserted into the slot 6. Upon insertion, the identification code conveyed by the barcode 7 is read into the unit's memory 8. The colour change in the reagent 4 is then determined by the reflectometry system 3 and the result also read into the unit's memory 8.

The patient is then prompted, by a message displayed on the telephone's display, to initiate a data call to the remote automatic telephone answering device 10. Following acceptance of the prompt by the user, the telephone 1 opens a data channel with a cellular telephone 12 network and transmits the read identification code to the automatic telephone answering device 10 via the telephone line 11. The answering device 10 responds by accessing the database 9 and downloading therefrom the calibration data corresponding to the received identification code. The answering device 10 then formats the calibration data and transmits it back to the mobile telephone 1 via the cellular network 12.

The calibration data received by the telephone 1 is stored in the memory 8. The telephone then proceeds to calculate a blood glucose test result using the measured colour change, the received calibration data, and a calculation algorithm which is pre-stored in the measurement unit's memory 8. This calculation is carried out by a central processing unit 13 of the phone 1.

The main steps associated with the measurement process at the measurement apparatus are shown in the flow diagram of Figure 2.

It will be appreciated that the same result can be obtained by transmitting the measured colour change from the measurement unit 2 and the mobile telephone 1, to the remote database 9, together with the identification code. The database 9 can then process the

measured value using the calibration data to generate a test result. It is then not necessary to transmit the calibration data to the measurement unit 2 and only the test result needs to be transmitted. The result received by the mobile telephone 1 can then be displayed directly on the telephone's display.

In addition to providing the measurement unit with the most up to date calibration data, the system described above provides a number of other significant advantages. For example, if a user mistakenly tries to use a test strip which has passed its 'use by' date, the user can be notified of this, and the measurement blocked, by the database and the automatic answering device. Accurate records can also be kept, by the database, of the usage pattern of test strips.

It will be appreciated that modifications may be made to the above described embodiment without departing from the scope of the present invention. For example, whilst the embodiment described above makes use of a mobile telephone, alternative embodiments may use land line telephones or two-way pagers.

Claims

1. A measurement system comprising:

measurement apparatus (1,2) for measuring a physical parameter using a consumable reagent (4), said reagent having an identification code (7) associated therewith;

automatic telephone answering means (10);

a database (9) coupled to the automatic telephone answering means (10) and containing a set of consumable reagent identification codes and respective calibration data;

means (1) for coupling the measurement apparatus to the automatic telephone answering means (1) via a telephone link (11,12),

the automatic telephone answering means (10) being arranged to receive an identification code transmitted from the measurement apparatus (1,2) over said telephone link (11,12), to subsequently access said database (9) to identify the calibration data associated with said transmitted identification code, and to either transmit the identified calibration data to the measurement apparatus (1,2), or to calculate a test result using the identified calibration data and transmit the test result to the measurement apparatus (1,2).

2. A system according to claim 1, wherein the automatic telephone answering means (10) is arranged to transmit calibration data associated with the received identification code back to the measurement apparatus (1,2) and the measurement apparatus (1,2) comprises data processing means (13) for processing said measured physical parameter, using the received calibration data, to provide a test result.

3. A system according to claim 1, wherein the measurement apparatus (1,2) is arranged to transmit said measured physical parameter to the automatic telephone answering means (10) together with said identification code, the system further comprising data processing means (9), coupled to the database (9) and the automatic telephone answering means (10), for calculating a test result using the identified calibration data and the received physical parameter, the automatic telephone answering means (10) being arranged to transmit the test result back to the measurement apparatus (1,2).

4. A system according to any one of the preceding claims, wherein the identification code is provided with the consumable reagent (4) in a machine readable form (7).

5. A system according to claim 4, wherein the identification code (7) is contained in a bar-code or in a solid state memory and the measurement apparatus (1,2) is provided with a machine reader (3) which is capable of reading the identification code into a memory (8) of the measurement apparatus (1,2).

6. A system according to any one of the preceding claims, wherein the measurement apparatus (1,2) comprises mobile telephone apparatus (1) which is able to connect the measurement apparatus (1,2) to the database (9) via a wireless transmission channel (12).

7. A system according to any one of the preceding claims, wherein the measurement apparatus (1,2) is arranged to provide a measure of the glucose level in a sample of a patient's blood.

8. A consumable reagent (4), or a pack of consumable reagents, in combination with an identification code (7), said reagent(s) (4) being for use with the measurement system of any one of the preceding claims, where said identification code (7) identifies a set of calibration data stored in said database (9).

9. The combination of claim 8, comprising a machine readable memory by means of which said identification code (7) is stored.

10. A method of deriving a test result from a physical parameter measured using a measurement apparatus and a consumable reagent, the method comprising;

providing a consumable reagent together with an identification code;

providing a database containing a set of identification codes and respective calibration data;

transmitting said identification code from the measurement apparatus to the database via a telephone link; and

automatically responding to receipt of the transmitted identification code by identifying the corresponding calibration data in the database, and transmitting that data, or a test result determined using the data, back to the measurement apparatus.

11. A method according to claim 10, said telephone link comprising a mobile telephone link, wherein the measurement apparatus comprises a measurement unit coupled to mobile telephone apparatus.

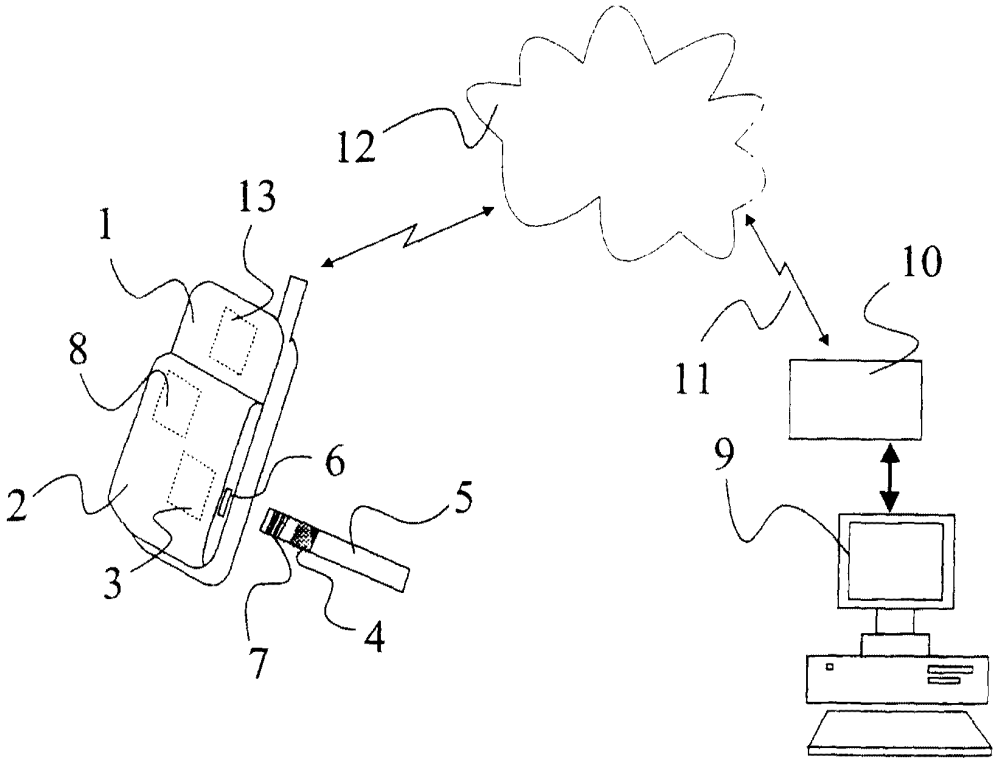


Figure 1

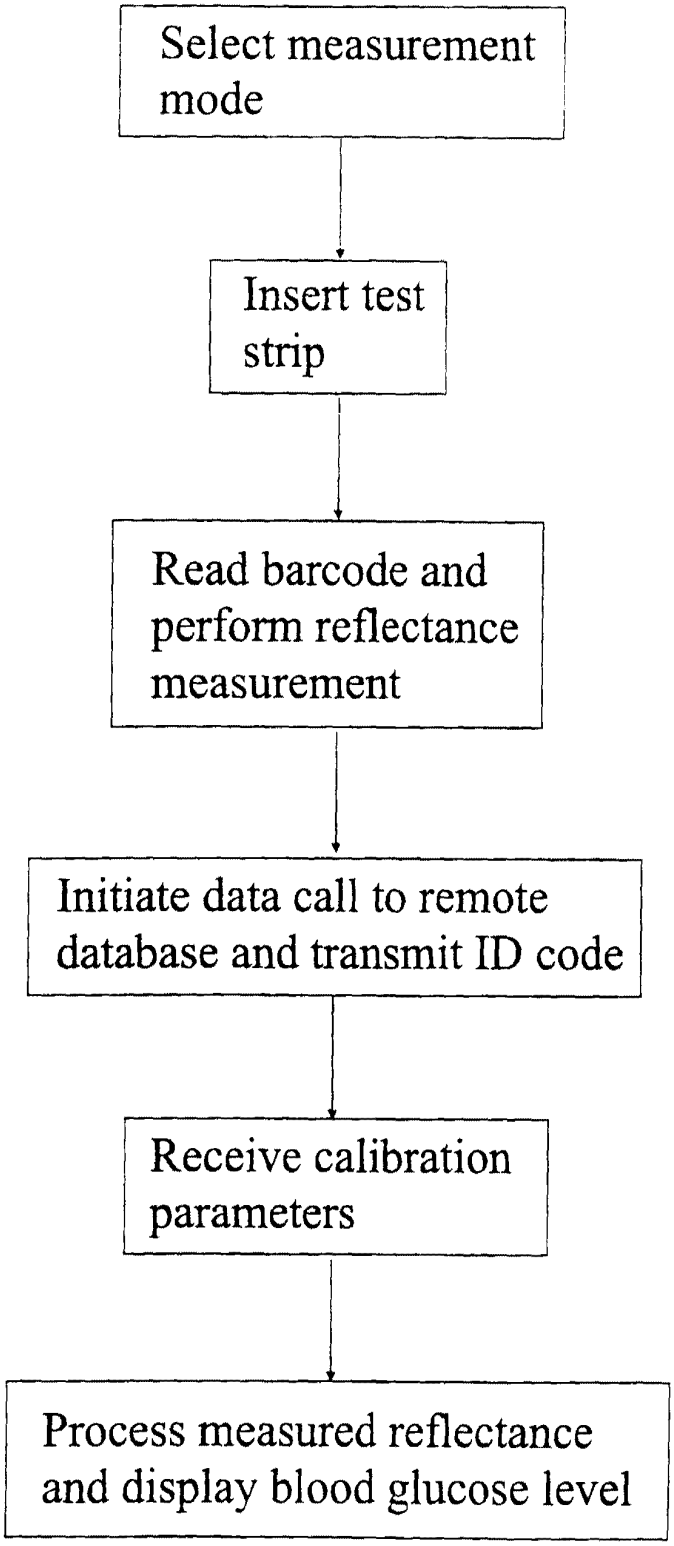

Select measurement mode
Insert test strip
Read barcode and perform reflectance measurement
Initiate data call to remote database and transmit ID code
Receive calibration parameters
Process measured reflectance and display blood glucose level

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 98/00740

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 33/487, A61B 5/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61B, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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A	EP 0807805 A2 (SOEHNLE-WAAGEN GMBH + CO), 9 May 1997 (09.05.97), abstract --	1-11
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 February 1999	17 -02- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Malin Keijser Telephone No. +46 8 782 25 00

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International application No.
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Information on patent family members

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(57) Abstract

Systems and methods for medical diagnosis or risk assessment for a patient are provided. These systems and methods are designed to be employed at the point of care, such as in emergency rooms and operating rooms, or in any situation in which a rapid and accurate result is desired. The systems and methods process patient data, particularly data from point of care diagnostic tests or assays, including immunoassays, electrocardiograms, X-rays and other such tests, and provide an indication of a medical condition or risk or absence thereof. The systems include an instrument for reading or evaluating the test data and software for converting the data into diagnostic or risk assessment information.

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POINT OF CARE DIAGNOSTIC SYSTEMS

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. application Serial No. 09/063,497, filed April 20, 1998, to Emory V. Anderson, Edward Nemec, Jerome Lapointe, Duane DeSieno, Ricardo Martinez, Gail Marzolf, Ronald Pong, Lynn Jones, Robert O. Hussa and Andrew Senyei, entitled "POINT OF CARE DIAGNOSTIC SYSTEMS, and to U.S. application Serial No. 09/017,901, filed February 3, 1998 to Emory V. Anderson, Edward Nemec, Jerome Lapointe, Duane DeSieno, Ricardo Martinez, Gail Marzolf, Ronald Pong, Lynn Jones, Robert O. Hussa and Andrew Senyei, entitled "POINT OF CARE DIAGNOSTIC SYSTEMS." For purposes of the United States, this application is a continuation-in-part of the above-identified applications.

This application is also related to U.S. application Serial No. 08/599,275 to Jerome Lapointe and Duane DeSieno, filed February 9, 1996, entitled "METHOD FOR DEVELOPING MEDICAL AND BIOCHEMICAL DIAGNOSTIC TESTS USING NEURAL NETWORKS", now abandoned, copending U.S. application Serial No. 08/798,306 to Jerome Lapointe and Duane DeSieno, filed February 7, 1997, entitled "METHOD FOR SELECTING MEDICAL AND BIOCHEMICAL DIAGNOSTIC TESTS USING NEURAL NETWORK-RELATED APPLICATIONS", and copending U.S. application Serial No. and 08/912,133 to Jerome Lapointe and Duane DeSieno, filed August 14, 1997, entitled "METHOD FOR SELECTING MEDICAL AND BIOCHEMICAL DIAGNOSTIC TESTS USING NEURAL NETWORK-RELATED APPLICATIONS"; and also copending U.S. application Serial No. 09/134,636, filed August 14, 1998. The subject matter of these applications is also described in International PCT application Nos. WO 97/29447 and PCT/US98/16891.

Where permitted, the subject matter of each of these patents and each of U.S. Application Serial Nos. 09/017,901, 08/599,275, 08/798,306 and 08/912,133 is herein incorporated herein by reference in its entirety. The

subject matter of published International PCT application No. International PCT application No. WO 97/29447, which corresponds to U.S. application Serial No. 08/912,133 is also herein incorporated in its entirety by reference thereto. U.S. Design Patent Applications Serial Nos. 29/086781 and 29/086799, each filed April 20, 1998, are also incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates to systems and methods that aid in providing a medical diagnosis or risk assessment for a patient using biochemical and historic patient data, including data from point of care diagnostic tests or assays, and processing the information to give an indication of a medical condition or risk.

BACKGROUND OF THE INVENTION

Evaluation of Immunoassay Data

In diagnostic immunochromatographic assays, where results are determined by a color change or the production of color, results are generally detected visually by human eye. As a result of the human perception and judgment involved, there is significant variance among those interpreting such test results as to whether a color change or other measurable signal has occurred, and the degree of such occurrence. Furthermore, there is a great deal of subjectivity involved in interpreting whether immunoassay results are positive or negative. This is particularly pronounced where the result is close to a threshold value. The variance is further enhanced when attempts are made to quantitate such assay test results. Accurate results may be critical for certain diagnostic assays.

It is desirable to develop techniques that are objective in nature, and that reduce the error associated with interpreting immunochromatographic and other assay test results. Therefore, it is an object herein to provide systems, methods, devices and instruments for objectively assessing data from biochemical and other tests and to use such data for diagnosis and risk assessment. It is also an object herein to incorporate decision-support methodologies into such systems and thereby enhance the diagnostic and risk assessment capabilities thereof.

It is also an object herein to provide systems and methods for use in detecting and measuring fetal fibronectin (fFN) levels in a patient sample and using such information to diagnose and assess risks of preterm labor, fetal membrane rupture and other related disorders and conditions of concern during pregnancy that may comprise the pregnancy or harm the newborn, including, for example, ascending genital infections such as chlamydia or herpes simplex virus.

SUMMARY OF THE INVENTION

Systems and methods for medical diagnosis or risk assessment for a patient are provided. These systems and methods are designed to be employed at the point of care, such as in emergency rooms, operating rooms, hospital laboratories and other clinical laboratories, doctor's offices, in the field, or in any situation in which a rapid and accurate result is desired. The systems and methods process patient data, particularly data from point of care diagnostic tests or assays, including immunoassays, chemical assays, nucleic acid assays, colorimetric assays, fluorometric assays, chemiluminescent and bioluminescent assays, electrocardiograms, X-rays and other such tests, and provide an indication of a medical condition or risk or absence thereof.

The systems include an instrument for reading or evaluating the test data and software for converting the data into diagnostic or risk assessment information. In certain embodiments, the systems include a test device, such as a test strip, optionally encased in a housing, for analyzing patient samples and obtaining patient data. In particular embodiments, the device includes a symbology, such as a bar code, which is used to associate identifying information, such as intensity value, standard curves, patient information, reagent information and other such information, with the test device. The reader in the system is optionally adapted to read the symbology.

Further, the systems optionally include a decision-support system or systems, such as a neural network, for evaluating the data, and also for subsequent assessment of the data, such as by integration with other patient information, including documents and information in medical records. All software and instrument components are preferably included in a single

package. Alternatively, the software can be contained in a remote computer so that the test data obtained at a point of care can be sent electronically to a processing center for evaluation.

The patient information includes data from physical and biochemical tests, such as immunoassays, and from other procedures. The test is performed on a patient at the point of care and generates data that can be digitized, such as by an electronic reflectance or transmission reader, which generates a data signal. The signal is processed using software employing data reduction and curve fitting algorithms, or a decision support system, such as a trained neural network, or combinations thereof, for converting the signal into data, which is used to aid in diagnosis of a medical condition or determination of a risk of disease. This result may be further entered into a second decision support system, such as a neural net, for refinement or enhancement of the assessment.

In a particular embodiment, systems and methods for detecting and measuring levels of a target analyte in a patient sample, analyzing the resulting data, and providing a diagnosis or risk assessment are provided. The systems and methods include an assay device in combination with a reader, particularly a computer-assisted reader, preferably a reflectance reader, and data processing software, preferably employing data reduction and curve fitting algorithms, optionally in combination with a trained neural network, for accurately determining the presence or concentration of analyte in a biological sample. In a preferred embodiment, the methods include the steps of performing an assay on a patient sample, reading the data using a reflectance reader and processing the reflectance data using data processing software. In a particular embodiment, the assay is an immunoassay. Preferred software includes data processing software employing data reduction and curve fitting algorithms, optionally in combination with a trained neural network, to determine the presence or amount of analyte in a given sample. The data obtained from the reader then can be further processed by the medical diagnosis system to provide a risk assessment or diagnosis of a medical condition as output. In alternative embodiments, the output can be used as input into a subsequent

decision support system, such as a neural network, that is trained to evaluate such data.

In a preferred embodiment, the assay device is a lateral flow test strip, preferably, though not necessarily, encased in a housing, designed to be read by the reader, and the assay is a sandwich immunoassay. For example, in one embodiment thereof, a patient sample is contacted with an antibody for a selected target analyte indicative of a disease, disorder or risk thereof. The antibody is preferably labeled by conjugation to a physically detectable label, and upon contacting with the sample containing the target analyte forms a complex. The antibody-analyte complex is then contacted with a second antibody for the antigen, which is immobilized on a solid support. The second antibody captures the antibody-analyte complex to form an antibody-analyte-antibody sandwich complex, and the resulting complex, which is immobilized on the solid support, is detectable by virtue of the label. The test strip is then inserted into a reader, where the signal from the label in the complex is measured. Alternatively, the test strip could be inserted into the reader prior to addition of the sample. Additionally, the housing may include a symbology, such as a bar code, which is also read by the reader and contains data related to the assay device and/or test run. The signal obtained is processed using data processing software, preferably employing data reduction and curve fitting algorithms, optionally in combination with a trained neural network, to give either a positive or negative result, or a quantitative determination of the concentration of analyte in the sample, which is correlated with a result indicative of a risk or presence of a disease or disorder. This result can optionally be input into a decision support system, and processed to provide an enhanced assessment of the risk of a medical condition as output. The entire procedure may be automated and/or computer-controlled.

In certain embodiments, the reflectance reader is adapted to read a symbology on the test device. The symbology is preferably a bar code, which be read in the same manner that the test strip in the device can be read. In these embodiments, the reader head scans across a bar code in a stepwise fashion. The data collected from the bar code is transformed into integrated

peak information and analyzed as alphanumeric characters, which are related to information related to the particular device and/or test run or other information, including patient information. Any bar code from among the many known in the in industry. In preferred embodiments, Code 39 (a trademark of Interface Mechanism, Inc., Lynnwood, WA; see, e.g., U.S. Patent No. 4,379,224, U.S. Patent No. 4,438,327, U.S. Patent No. 4,511,259 or Code 128 bar codes (see, e.g., U.S. Patent No. 5,227,893) are used.

In a particular embodiment, the analyte to be detected is fetal fibronectin (fFN) and the result obtained is a positive or negative indication of pregnancy or the risk of certain pregnancy-related conditions or fertility and infertility-related conditions, including ectopic pregnancy, preterm labor, pre-eclampsia, imminent delivery, term induction and fetal membrane rupture and infections of concern during pregnancy that may comprise the pregnancy or harm the newborn, including, for example, ascending genital infections such as chlamydia or herpes simplex virus. Thus, provided herein is a rapid fFN test using a lateral flow test device.

At the very least, this test provides the same clinically relevant information as a fFN ELISA (an enzyme linked immunosorbent sandwich assay (ELISA)) test heretofore available in significantly less time and at the point of care. The fFN immunoassay provided herein allows the user to test a cervicovaginal swab sample in about 20 minutes. When practiced as described herein, additional information, such as a more accurate risk assessment or diagnosis, can be obtained.

The system herein provides a means to detect and to quantitate concentrations of fFN throughout pregnancy and to assess the risk and detect conditions associated therewith. Because of the sensitivity of the combination of the reader and devices provided herein, fFN may be monitored throughout pregnancy, including times when it is not detected by less sensitive systems.

The reflectance reader and test strip device are also provided herein. Also provided herein are the neural nets for assessing the data.

A method for classifying an image is also provided. The method includes the steps of reducing the image to a set of derived parameters from which the image can be reconstructed within a predetermined degree of tolerance; inputting the derived parameters into a classification neural network; and determining the classification of the image based on the output of the classification neural network. The method of reducing the image to a set of derived parameters is achieved by defining a mathematical function that contains a plurality of parameters representative of the image; and optimizing the parameters of the function using a methodology that minimizes the error between the image and a reconstruction of the image using the function.

In an alternative embodiment, the method of reducing the image to a set of derived parameters is achieved by inputting the image into a trained neural network, where the inputs to the network represent the image, the hidden layer of the network is such that the number of hidden elements is smaller than the number of inputs to the network, and the outputs of the network represent reconstruction of the image; and setting the derived parameters to the output values of the trained neural network.

In another alternative embodiment, the method of reducing the image to a set of derived parameters is achieved by defining a neural network in which the inputs to the network are the coordinates of a point in the image, the hidden layer contains a plurality of elements, and the output of the network represents the reconstruction of the associated point in the image; training the neural network so that the error between the network output and the image are minimized for all points in the image; and setting the derived parameters to the weights of the hidden layer of the trained neural network.

The neural networks and computer systems used in the methods are also provided.

Also provided are methods for assessing the validity of diagnostic test results and for a method for developing controls for assays. The method of developing a control for any assay system includes the steps of analyzing a plurality of test runs of the assay and obtaining results of the test runs;

classifying the results by type of result; and identifying a pattern representative of or predictive of each type of result. When the assay is run the results therefrom are compared to the representative pattern, and, if the results vary from the pattern, they can be discarded. The pattern and classification can be effected by any decision support system, including curve fitting routines and neural nets. For example, data from an assay are fit to to a predetermined curve based on expected results for a predetermined number of iterations. If the curve for a particular run of the assay deviates beyond an acceptable predetermined fit, the data from the assay is rejected. The curve data can be generated for any type of test and typically will encompass at least variables. For example, for the fFN assay provided herein a curve or function for intensity of the reading versus the distance traveled on the strip (generally normalized) has been generated based on numerous runs of the assay. For any given run of the assay, if the curve of intensity versus distance varies by a selected predetermined amount, it indicates that the assay did not run properly, and the results should be discarded. If the results match the curve, then this indicates that the assay ran properly. This type of curve fitting can be performed for any assay or test and can use typically at least two and up to any number of variables or parameters for generating the curve. This may generated and the control step performed using a neural network or other decision support system.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a top view of an assay test strip, such as an immunoassay test strip;

FIG. 1B is a side view of the assay test strip of FIG. 1;

FIG. 2A is a perspective view of an assay device, including the assay test strip of FIG. 1A and FIG. 1B and housing assembly and showing a bar code, which can optionally be affixed to the housing;

FIG. 2B is a perspective view of an alternative embodiment of an assay device, including the assay test strip of FIG. 1A and FIG. 1B and housing assembly and showing a bar code, which can optionally be affixed to the housing;

FIG. 3 is a perspective view of the assay device of FIG. 2B showing the individual components of the device;

FIG. 4 is a top view of an exemplary housing assembly for the assay test strip of FIG. 1;

FIG. 5 is a side assembly view of the housing assembly of FIG. 4;

FIG. 6 is a top view of an embodiment of an assay reader and an assay device, inserted therein, in accordance with an exemplary embodiment of the reader;

FIG. 7 is a perspective view of portion of the assay device of FIG. 2A shown inserted into a cassette slot of a lower housing and extending to a reader head assembly within an exemplary embodiment of an assay reader;

FIG. 8 is a top view of the lower housing of the assay reader of FIG. 7 with the assay device inserted therein and a stepper motor shown positioned relative to the assay device as is when the assay device is fully inserted into the cassette slot of the reader;

FIG. 9 is a side view of the lower housing of the reader device of FIG. 7 with the assay device of FIG. 2A fully inserted with the stepper motor shown positioned relative to the fully inserted assay device, with a reader head shown positioned in a lowered position over a test opening of the assay device, and with a carriage wheel shown engaged by the assay device so as to lower the reader head into its lowered position therein;

FIG. 10 is a side view of a reader head assembly such as is found in the reader device of FIG. 6;

FIG. 11 is a side view of a reader head of the reader head assembly of FIG. 10;

FIG. 12 is a reverse angle side view of the reader head assembly of FIG. 10;

FIG. 13 is a reverse angle side view of the reader head of FIG. 11;

FIG. 14 is a side view of the reader head assembly of FIG. 10, having been actuated so as to pivot the reader head assembly into a raised position suitable for insertion and removal of the assay device into and from the reader head assembly within the assay reader;

FIG. 15 is an end view of the reader head of FIG. 11;

FIG. 16 is an end view of the reader head assembly of FIG. 10;

FIG. 17 is a cut-away view of the reader head assembly of FIG. 11 with first and second light emitting diodes, a photodetector, corresponding fiberoptic bundles and an aperture at a lower end thereof depicted;

FIG. 18 is a partial closeup cross-sectional view of a reader head tip of the reader head of FIG. 17 showing the aperture and ends of fiberoptic fibers of the fiberoptic bundles of FIG. 17;

FIG. 19 is a closeup bottom view of the aperture of the reader head of FIG's. 17 and 18 illustrating a sigmoidal pattern for positioning individual fiberoptic fibers (fiberoptic conductors);

FIG. 20 is a closeup end view of the corresponding fiberoptic bundle at the first light emitting diode of FIG. 17 from which the fiberoptic bundle conducts light from the first light emitting diode;

FIG. 21 is a schematic diagram illustrating a process by which an assay test strip is analyzed so as to determine an amount of background light at a control region of the assay test strip;

FIG. 22 is a schematic diagram illustrating a process by which an assay test strip is analyzed so as to determine an amount of reflection resulting from a first illumination of a control portion of the assay test strip; and

FIG. 23 is a schematic view diagram illustrating a process by which an assay test strip is analyzed so as to determine an amount of reflection resulting from a second illumination of a control portion of the assay test strip;

FIG. 24 is a side view of an exemplary embodiment of the reader that is adapted for reading a bar code; and

FIG. 25 is an example of a bar code in accordance with an exemplary embodiment of the assay device.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein

are, unless noted otherwise, incorporated by reference in their entirety. In the event a definition in this section is not consistent with definitions elsewhere, the definition set forth in this section will control.

As used herein, point of care testing refers to real time diagnostic testing that can be done in a rapid time frame so that the resulting test is performed faster than comparable tests that do not employ this system. For example, the exemplified fFN immunoassay, is performed in less time than the fFN ELISA assay (i.e., less than about 3 to 4 hours, preferably less than 1 hour, more preferably less than half an hour). In addition, with the method and devices provided herein, it can be performed rapidly and on site, such as in a doctor's office, at a bedside, in a stat laboratory, emergency room or other such locales, particularly where rapid and accurate results are required. The patient can be present, but such presence is not required. Point of care includes, but is not limited to: emergency rooms, operating rooms, hospital laboratories and other clinical laboratories, doctor's offices, in the field, or in any situation in which a rapid and accurate result is desired.

As used herein, an anti-fFN antibody is an antibody that binds selectively with fFN. Such antibodies are known to those of skill in the art and also may be readily isolated.

As used herein, a test strip refers to any means on which patient test data or other data is generated, recorded or displayed in a manner that forms an image or from which an image can be generated. Such strips, include, but are not limited to, immunochromatographic test strips, such as lateral flow devices, X-ray films, such as X-rays and films produced from sequencing gels, EKG printouts, MRI results and other such means that generate or from which an image as defined herein can be generated. The strip is preferably adapted for scanning or reading by a reader, preferably the reader provided herein. Although referred to as a "strip", it can be of any shape or geometry, including rectangular, three dimensional, circular, and so forth.

As used herein, a sigmoidal pattern (also referred to herein as sigmoidal-like; see, e.g., FIG. 19) with reference to the fiberoptics refers to the S-shaped or snake-like pattern of illumination selected for maximizing illumination across

the lines on the test strip. The pattern is not strictly a sigmoidal shape, but refers to a pattern such as that depicted in FIG. 19, which pattern provides a means for adding more area to any reading. Any other pattern that achieved this result is encompassed within this expression.

As used herein, quantitative results are results that are absolute or relative values; qualitative results are typically negative or positive type results.

As used herein, fetal restricted antigens refers to antigen that are present in pregnant women uniquely, or in substantially elevated amounts compared to non-pregnant women in maternal serum, plasma, urine, saliva, sweat, tears and other bodily fluids.

As used herein, fetal fibronectin is a fetal restricted antigen found in placenta, amniotic fluid and fetal connective tissue. It differs structurally from adult fibronectins. Fetal fibronectin is not present in significant quantities in maternal plasma or serum. Fetal fibronectin may be captured with a general binding antibody, such as an anti-fibronectin antibody, or an anti-fetal restricted antigen antibody, such as anti-fetal fibronectin antibody.

As used herein, an immunoassay is defined as any method using a preferential binding of an antigen with a second material, a binding partner, usually an antibody or another substance having an antigen binding site, which binds preferentially with an epitope of the fetal restricted antigen. Preferential binding, as used herein, refers to binding between binding partners that is selective and generally specific, and demonstrates less than 10%, preferably less than 5%, cross-reactive nonspecific binding. The immunoassay methods provided herein include any known to those of skill in the art, including, but not limited to, sandwich, competition, agglutination or precipitation, for example.

As used herein, a solid support refers to the material to which the antibody is linked. A variety of materials can be used as the solid support. The support materials include any material that can act as a support for attachment of the molecules of interest. Such materials are known to those of skill in this art. These materials include, but are not limited to, organic or inorganic polymers, natural and synthetic polymers, including, but not limited to, agarose, cellulose, nitrocellulose, cellulose acetate, other cellulose derivatives, dextran,

dextran-derivatives and dextran co-polymers, other polysaccharides, glass, silica gels, gelatin, polyvinyl pyrrolidone, rayon, nylon, polyethylene, polypropylene, polybutlyene, polycarbonate, polyesters, polyamides, vinyl polymers, polyvinylalcohols, polystyrene and polystyrene copolymers, polystyrene cross-linked with divinylbenzene or the like, acrylic resins, acrylates and acrylic acids, acrylamides, polyacrylamides, polyacrylamide blends, co-polymers of vinyl and acrylamide, methacrylates, methacrylate derivatives and co-polymers, other polymers and co-polymers with various functional groups, latex, butyl rubber and other synthetic rubbers, silicon, glass, paper, natural sponges, insoluble protein, surfactants, red blood cells, metals, metalloids, magnetic materials, or other commercially available media.

As used herein, a reader refers to an instrument for detecting and/or quantitating data, such as on test strips. The data may be visible to the naked eye, but does not need to be visible.

As used herein, a reflectance reader refers to an instrument adapted to read a test strip using reflected light, including fluorescence, or electromagnetic radiation of any wavelength. Reflectance can be detected using a photodetector or other detector, such as charge coupled diodes (CCD). A preferred reflectance reader, which is provided and described herein, includes a cassette slot adapted to receive a test-strip, light-emitting diodes, optical fibers, a sensing head, including means for positioning the sensing head along the test strip, a control circuit to read the photodetector output and control the on and off operation of the light-emitting diodes, a memory circuit for storing raw and/or processed data, and a photodetector, such as a silicon photodiode detector.

As used herein, a sensing head refers to the assembly which is adapted to read a test strip using reflected light or other electromagnetic radiation. Thus, the sensing head in the reader provided herein refers to the part of the sensing head assembly that randomizes the optical bundles and arranges the fibers in the plane normal to the test strip.

As used herein, color refers to the relative energy distribution of electromagnetic radiation within the visible spectrum. Color can be assessed visually or by using equipment, such as a photosensitive detector.

As used herein, a color change refers to a change in intensity or hue of color or may be the appearance of color where no color existed or the disappearance of color.

As used herein, a decision-support system, also referred to as a "data mining system" or a "knowledge discovery in data system", is any system, typically a computer-based system, that can be trained on data to classify the input data and then subsequently used with new input data to make decisions based on the training data. These systems include, but are not limited, expert systems, fuzzy logic, non-linear regression analysis, multivariate analysis, decision tree classifiers, Bayesian belief networks and, as exemplified herein, neural networks.

As used herein, an adaptive machine learning process refers to any system whereby data are used to generate a predictive solution. Such processes include those effected by expert systems, neural networks, and fuzzy logic.

As used herein, an expert system is a computer-based problem solving and decision-support system based on knowledge of its task and logical rules or procedures for using the knowledge. The knowledge and the logic are entered into the computer from the experience of human specialists in the area of expertise.

As used herein, a neural network, or neural net, is a parallel computational model comprised of densely interconnected adaptive processing elements. In the neural network, the processing elements are configured into an input layer, an output layer and at least one hidden layer. Suitable neural networks are known to those of skill in this art (see, e.g., U.S. Patents 5,251,626; 5,473,537; and 5,331,550, Baxt (1991) "Use of an Artificial Neural Network for the Diagnosis of Myocardial Infarction," Annals of Internal Medicine 115:843; Baxt (1992) "Improving the Accuracy of an Artificial Neural Network Using Multiple Differently Trained Networks," Neural Computation 4:772; Baxt

(1992) "Analysis of the clinical variables that drive decision in an artificial neural network trained to identify the presence of myocardial infarction," Annals of Emergency Medicine 21:1439; and Baxt (1994) "Complexity, chaos and human physiology: the justification for non-linear neural computational analysis," Cancer Letters 77:85).

As used herein, a processing element, which may also be known as a perceptron or an artificial neuron, is a computational unit which maps input data from a plurality of inputs into a single binary output in accordance with a transfer function. Each processing element has an input weight corresponding to each input which is multiplied with the signal received at that input to produce a weighted input value. The processing element sums the weighted inputs values of each of the inputs to generate a weighted sum which is then compared to the threshold defined by the transfer function.

As used herein, a transfer function, also known as a threshold function or an activation function, is a mathematical function which creates a curve defining two distinct categories. Transfer functions may be linear, but, as used in neural networks, are more typically non-linear, including quadratic, polynomial, or sigmoid functions.

As used herein, an image is a multi-dimensional array of data points, where each data point is represented by a number, or a set of numbers, and where there is a relationship between adjacent points in each of the dimensions. The index values in each dimension typically represent a linear relationship, like position or time, but are not limited to these types of relationships. A single digitized scan line from a TV frame would be considered a two dimensional image. In the case of the preferred embodiment, an image refers to a one-dimensional set of pixels, which encode the intensity of the color on the test strip.

As used herein, classifying an image refers to associating an object or state with the image. Images of fruit might be classified as to the type of fruit shown in the image. In the case of the preferred embodiment, classifying the test strip image refers to associating the positive or negative state with the image.

As used herein, reconstructing an image refers to producing an image from a mathematical function. When an image is represented by a mathematical function, there may be errors in the representation due to any number of factors.

As used herein, backpropagation, also known as backprop, is a training method for neural networks for correcting errors between the target output and the actual output. The error signal is fed back through the processing layer of the neural network, causing changes in the weights of the processing elements to bring the actual output closer to the target output.

As used herein, Quickprop is a backpropogation method that was proposed, developed and reported by Fahlman ("Fast Learning Variations on Back-Propagation: An Empirical Study", Proceedings on the 1988 Connectionist Models Summer School, Pittsburgh, 1988, D. Touretzky, et al., eds., pp.38-51, Morgan Kaufmann, San Mateo, CA; and, with Lebriere, "The Cascade-Correlation Learning Architecture", Advances in Neural Information Processing Systems 2,(Denver, 1989), D. Touretzky, ed., pp. 524-32. Morgan Kaufmann, San Mateo, CA).

As used herein, diagnosis refers to a predictive process in which the presence, absence, severity or course of treatment of a disease, disorder or other medical condition is assessed. For purposes herein, diagnosis will also include predictive processes for determining the outcome resulting from a treatment.

As used herein, risk refers to a predictive process in which the probability of a particular outcome is assessed.

As used herein, a patient or subject includes any mammals for whom diagnosis is contemplated. Humans are the preferred subjects.

As used herein, biochemical test data refers to data from any analytical methods, which include, but are not limited to: immunoassays, bioassays, including nucleic acid and protein based assays, chromatography, data from monitors, and imagers; measurements and also includes data related to vital signs and body function, such as pulse rate, temperature, blood pressure, data generated by, for example, EKG, ECG and EEG, biorhythm monitors and other

such information. The analysis can assess for example, chemical analytes, serum markers, antibodies, protein, nucleic acids and other such material obtained from the patient through a sample. Immunoassays are exemplified herein, but such exemplification is not intended to limit the intended scope of the disclosure, which is applicable to any test strip and test data read by an instrument, particularly a reflectance reader.

As used herein, patient historical data refers to data obtained from a patient, such as by questionnaire format, but typically does not include biochemical test data as used herein, except to the extent such data is historical, a desired solution is one that generates a number or result whereby a diagnosis of a disorder can be generated.

As used herein, a run is defined as a group of tests that include a at least one of a positive reference, positive control, negative control and any number of clinical samples within a 24 hr. period.

As used herein, symbology refers to a code, such as a bar code, that is engraved or imprinted on the test device. The symbology is any code known or designed by the user. The symbols are associated with information stored in a remote computer or memory or other such device or means. For example, each test device can be uniquely identified with an encoded symbology. It is contemplated herein that identifying and other information can be encoded in the bar code, which can be read by the reader when the test strip is read. Alternatively, the bar code or other symbology may be read by any of reading device known to those of skill in the art.

As used herein, a bar code is a symbology, typically a field of alternating dark bars and reflective spaces of varying widths, that is affixed onto or associated with an item and provides identifying information about the item. Bar codes can placed on a reflective background, and the contrast between the dark bars and reflective spaces, or the reflectivity ratio, allows an optical sensor in a reader to discern the transitions between the bars and spaces in the symbol. Bar codes are electro-optically scanned, typically using a laser or LED, and generate a signal that is transmitted to an associated computer whose memory has digitally stored therein identifying information associated with the

item. The item is thereby automatically identified by its bar code and can be tracked, or additional information can be added to the stored information associated with the encoded item.

Several bar code formats are available and are used for different purposes and can be adapted for use in the systems herein or new ones, which can be one or two dimensional or more, devised. For example, a number of different bar code symbologies exist, these symbologies include UPC/EAN codes, Code 39, Code 128, Codeabar, Interleaved 2 of 5 and many others; two-dimensional codes, such as PDF 417, Code 49, Code 16K; matrix codes (Data Code, Code 1, Vericod); graphic codes; and any others known to those of skill in the art. Preferred herein are one-dimensional codes, such as the well known Code 39 and Code 128, although two-dimensional codes (see, e.g., U.S. Patent Nos. 5,243,655 and 5,304,786, are also suitable for use herein.

The 39 bar code provides a fully alphanumeric bar code for data entry systems. This bar code is especially effective in applications that use alphanumeric data for item identification. The structure of 39 permits it to be printed by a wide variety of techniques, including offset, letterpress, fully-formed impact printers, dot matrix printers, and on-impact printing devices. Code 39 is the most widely used alphanumeric bar code. It has been accepted as a standard code by many companies and industries. Specification ANSI Draft MH10.X-1981, entitled, "Specifications for Bar Code Symbols on Transport Packages & Unit Loads," describes three different bar code symbologies. Code 39 is called 3-of-9 code in the ANSI specification. Moreover, the Depae MIL-STD-1189, dated Jan. 4, 1982, defines 39 (called 3 of 9 code) as the standard symbology for marking unit packs, outer containers, and selected documents.

Code 39 includes 9 bits, at least three of which are always 1. Code 39 can be used to encode a set of 43 characters, including upper case alphabetic and numeric (0-9) characters, as well as seven special characters (-, ., , *, $, /, + and %). The beginning and end characters are always an asterisk (*). The code uses narrow and wide bars along with narrow and wide spaces, and the encoding for a single character is made up of a pattern of bars and spaces. The

code structure is three wide elements out of a total of nine elements, where an element is the area occupied by a bar or space). The nine elements include five bars and four spaces.

In Code 128, every character is constructed of eleven bars and spaces, and all 128 ASCII characters, i.e., numeric characters, upper and lower case characters, punctuation and control codes are encoded. There are three different character sets to select from: one set encodes all upper case characters and all ASCII control characters; another encodes all upper and lower case characters; and the third encodes all numeric characters. Through the use of special characters, it is possible to switch between character sets within a single code symbol. Code 128 uses four different bar and space widths. Each data character encoded in a Code 128 symbol is made up of 11 black or white modules. Three bars and three spaces are formed out of the 11 modules. There are 106 different three bar/three space combinations. Bars and spaces can vary between one and four modules wide. The stop character is made up of 13 modules. The symbol includes a quiet zone (10 x-dimensions), a start character, the encoded data, a check character, the stop character and a trailing quiet zone (10 x-dimensions) (see, e.g., U.S. Patent No. 5,262,625).

Systems for generating and reading bar codes are readily available and are well known in the art.

Point of Care Diagnostic and Risk Assessment Systems

Provided herein are systems for use at the point of care for diagnosing and assessing certain medical risks. The systems are designed for use on site at the point of care, where patients are examined and tested, and for operation remote from the site.

The systems are designed to accept input in the form of patient data, including, but not limited to biochemical test data, physical test data, historical data and other such data, and to process and output information, preferably data relating to a medical diagnosis or a disease risk indicator. The patient data may be contained within the system, such as medical records or history, or may be input as a signal or image from a medical test or procedure, for example, immunoassay test data, blood pressure reading, ultrasound, X-ray or MRI, or

introduced in any other form. Specific test data can be digitized, processed and input into the medical diagnosis expert system, where it may be integrated with other patient information. The output from the system is a disease risk index or medical diagnosis.

In a preferred embodiment, the system includes a reader, such as a reflectance or transmission reader, preferably a reflectance reader, for reading patient data, a test device designed to be read in the reader, and software for analysis of the data. In an exemplified embodiment of the system, the reader is the reflectance reader provided herein. A test strip device in a plastic housing designed for use with the reader, optionally including a symbology, such as an alphanumeric character bar code or other machine-readable code, and software designed for analysis of the data generated from the test strip are also provided.

The system is described with reference to an exemplary test for fFN, but it is to be understood that this embodiment is exemplary, and that the system, particularly the system with the reader head, can be adapted for any suitable assay.

Assays

Any assay is intended for use in the systems and methods herein. Such assays include, but are not limited to: nucleic acid detection, including using amplification and non-amplification protocols, any assay that relies on colorimetric or spectrometric detection, including fluorometric, luminescent detection, such as creatine, hemoglobin, lipids, ionic assays, blood chemistry. Any test that produces a signal, or from which a signal can be generated, that can be detected by a detector, such as a photodetector or a gamma counter, is intended for use as part of the systems provided herein. Any wavelength is intended to be included.

Immunoassays, including competitive and non-competitive immunoassays, are among those preferred for determination of the presence or amount of analyte in a patient sample, and are exemplified herein. It is understood that immunoassays are provided for exemplification, and that the methods and systems provided herein have broad applicability to patient test data and other test data.

A number of different types of immunoassays using a variety of protocols and labels are well known. Immunoassays may be homogeneous, i.e. performed in a single phase, or heterogeneous, where antigen or antibody is linked to an insoluble solid support upon which the assay is performed. Sandwich or competitive assays may be performed. The reaction steps may be performed simultaneously or sequentially. Threshold assays may be performed, where a predetermined amount of analyte is removed from the sample using a capture reagent before the assay is performed, and only analyte levels of above the specified concentration are detected. Assay formats include, but are not limited to, for example, assays performed in test tubes, wells or on immuno-chromatographic test strips, as well as dipstick, lateral flow or migratory format immunoassays.

Any known immunoassay procedure, particularly those that can be adapted for use in combination with lateral flow devices as described herein, can be used in the systems and methods provided herein.

Test Device

Any device that is compatible for use with a reader, preferably a reflectance reader, for determining the assay result is contemplated for use herein. Examplary devices include test strips, such as lateral flow devices, preferably contained within a housing adapted for use with the reader.

Any such test strips that can be adapted for use in combination with a reader are contemplated for use in the systems provided herein. Such test strip devices as are known to those of skill in the art (see, e.g., U.S. Patent Nos. 5,658,801, 5,656,502, 5,591,645, 5,500,375, 5,252,459, 5,132,097 and many other examples) may be used in systems as described herein, particularly in combination with the reader provided herein.

Typically these test devices are intended for use with biological samples, such as, for example, but not limited to, saliva, blood, serum, cerebral spinal fluid, cervico-vaginal samples. Other biological samples, such as food samples, which are tested for contamination, such as by bacteria or insects, are also contemplated.

Target analytes include, but are not limited to: nucleic acids, proteins, peptides, such as human immunodeficiency virus (HIV) antigens, antigens indicative of bacterial, such as Salmonella and E. coli, yeast or parasitic infections, apolipoprotein(a) and lipoprotein(a), environmental antigens, human chorionic gonadotropin (hCG), E-3-G, interleukins and other cytokines and immunomodulatory proteins, such as IL-6 and interferon, small nuclear ribonuclear particles (snRNP) antigens, fFN and other indicators, such as IGF binding protein-1, of pregnancy related disorders. Immunoassay and other tests for such antigens are known (see, *e.g.*, U.S. Patent Nos. 4,919,889, 5,079,171, 5,096,830, 5,185,270, 5,223,440, 5,236,846, 5,281,522, 5,096,830 and 5,079,171; see, also International PCT application No. WO 93/09438 and U.S. Patent No. 5,516,702; International PCT application No. WO 92/12426 and U.S. Patent No. 5,554,504; and see also International PCT application No. WO 94/17405 or EP A 0 680 607, and other such tests known to those of skill in the art.

Immunoassay Test Strip

Any suitable test strip or medium for performing an assay is contemplated herein as long as the "strip" can be adapted for use with a reader as described herein. A preferred embodiment is an immunoassay test strip that includes a membrane system that defines a liquid flow pathway.

For performing immunoassays, lateral flow test immunoassay devices are among those preferred herein. In such devices, a membrane system forms a single fluid flow pathway along the test strip. The membrane system includes components that act as a solid support for immunoreactions. For example, porous or bibulous or absorbent materials may be placed on a strip such that they partially overlap, or a single material can be used, in order to conduct liquid along the strip. The membrane materials may be supported on a backing, such as a plastic backing. In a preferred embodiment, the test strip includes a glass fiber pad, a nitrocellulose strip and an absorbent cellulose paper strip supported on a plastic backing.

Antibodies that react with the target analyte and/or a detectable label system are immobilized on the solid support. The antibodies may be bound to

the test strip by adsorption, ionic binding, van der Waals adsorption, electrostatic binding, or by covalent binding, by using a coupling agent, such as glutaraldehyde. For example, the antibodies may be applied to the conjugate pad and nitrocellulose strip using standard dispensing methods, such as a syringe pump, air brush, ceramic piston pump or drop-on-demand dispenser. In a preferred embodiment, a volumetric ceramic piston pump dispenser is used to stripe antibodies that bind the analyte of interest, including a labeled antibody conjugate, onto a glass fiber conjugate pad and a nitrocellulose strip.

The test strip may or may not be otherwise treated, for example, with sugar, a polymer such as polyvinylpyrrolidone or other release agent to facilitate mobility along the test strip, or with water-soluble non-immune animal proteins, such as albumins, including bovine (BSA), other animal proteins, water-soluble polyamino acids, or casein to block non-specific binding sites.

An exemplary immunoassay test strip provided herein is shown in FIG. 1. The test strip is described in detail in EXAMPLE 1. This test strip is provided for purposes of exemplification of the methods and systems provided herein and is not intended to limit the application to immunoassay test strip devices nor to the exemplified device.

Test Strip Housing

The test strip or other such medium optionally may be contained within a housing suitable for reading, such as for insertion into a reader, such as the reflectance reader provided herein. The housing may be fabricated from any suitable material, including plastic or other inert material that does not interfere with the assay procedure. An exemplary assay device, including a test strip and housing assembly, is shown in FIGs. 2-5.

If desired for tracking and identification, the test strip housing or other portion thereof can include an identifying or identifiable symbology, such as a bar code. The bar code can contain information that can be associated with data related to the assay device, patient data, test run, lot numbers, calibration information, and any information.

For example, information associated with the device, such as lot number, expiration date, analyte and intensity value, or information related to the test run, such as date, reflectance value or other such information, can be encoded and associated, such as in a database with a bar code imprinted on the device. Any bar code system that provides the appropriate line thickness and spacing can be used. Code 39 and Code 128 are among the bar code systems suitable for use in the systems herein. Other one dimensional or multidimensional (i.e., two or three dimensional coding systems can be used or developed).

In an exemplary embodiment, Code 39 is used, and one exemplary bar code is depicted in FIG. 25. The bar code contains 11 alphanumeric characters, including 2 alphabetic and 9 numeric characters. The first and last characters are asterisks (*), as is standard in the Code 39 system. The lot number is stored as 1 alpha and 4 numeric codes so that product complaints or questions can be traced to a particular lot number. In the exemplified embodiment, the first character represents the month of production, the second is a digit representing the year of production and the last three are an index value indicating the lot number. Thus, the lot number "A8001" represents the first device in a lot produced in January, 1998. The next two characters ("01") represent the identity of the analyte as 2 numerics (00-99). This permits the use of up to 100 different analytes with the system. A reflectance intensity value (00-99), which sets the reference threshold for which controls and patient samples can be compared, is stored as the next two numeric characters ("01"). This eliminates the need to run liquid reference samples on a daily basis. FIGs. 2 and 3 depict assay devices that optionally include bar codes, **216** and **316**, respectively. In the exemplified embodiment, the cassette expiration date is stored as 1 alpha and 1 numeric code to prevent the use of expired devices. In the example given, an expiration code of "A9" represents an expiration date of January, 1999. Other codes can be readily employed and other information, as desired, encoded thereon.

Antibodies

Any antibodies, including polyclonal or monoclonal antibodies, including animal, such as rodent, humanized, or any fragment thereof, such as Fab fragments, that bind the analyte of interest, are contemplated for use herein. Monoclonal and/or polyclonal antibodies may be used. The antibody is selected for the particular target antigen.

For example, for the exemplified assay, anti-fetal fibronectin antibodies are employed. A mouse monoclonal anti-fetal fibronectin antibody has been used in a labeled antibody-conjugate for detecting fetal fibronectin, and a polyclonal goat anti-mouse antibody used to bind fetal fibronectin to form a sandwich complex. In the exemplary embodiment, an antibody that binds to the labeled antibody conjugate that is not complexed with fetal fibronectin is immobilized on the test strip and used as a control antibody. Such antibodies are known to those of skill in the art (see, *e.g.*, U.S. Patent Nos. 5,281,522; 4,894,326 and 5,243,029,) and are discussed below.

Conjugation of the Antibody to a Label

An antibody conjugate containing a detectable label may be used to bind the analyte of interest. The detectable label used in the antibody conjugate may be any physical or chemical label, such as fluorescent, chemiluminescent, bioluminescent, radio-, and enzyme labels, capable of being detected, directly or indirectly, on a solid support using a reader, preferably a reflectance reader, and capable of being used to distinguish the reagents to be detected from other ompounds and materials in the assay. Suitable antibody labels are well known to those of skill in the art. The labels include, but are not limited to enzyme-substrate combinations that produce color upon reaction, colored particles, such as latex particles, colloidal metal or metal or carbon sol labels, fluorescent labels, and liposome or polymer sacs, which are detected due to accumulation of the label. A preferred label is a colored latex particle, which are well known and commericially available. In an alternative embodiment, colloidal gold is used in the labeled antibody conjugate.

The label may be derivatized for linking antibodies, such as by attaching functional groups, such as carboxyl groups to the surface of a particle to permit covalent attachment of antibodies. Antibodies may be conjugated to the label using well known coupling methods. Coupling agents such as glutaraldehyde or carbodiimide may be used. The labels may be bonded or coupled to the antibodies by chemical or physical bonding. In a preferred embodiment, a carbodiimide coupling reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), is used to link antibodies to latex particles. Coupling reagents are well-known to those of skill in this art, and any such agent may be used. Latex particles are commercially available and also well known (see, *e.g.*, U.S. Patent Nos. 4,373,932, 4,436,826 and 4,716,123).

Measurement of Analytes

Any analyte that can be detected in any assay, particularly colorimetric assays, including immunoassays, and that can be associated with a disorder is contemplated for as a target herein. Suitable analytes are any which can be used, along with a specific binding partner, such as an antibody, or a competitor, such as an analog, in an assay. Analytes may include, but are not limited to proteins, haptens, immunoglobulins, enzymes, hormones (e.g., hCG, LH, E-3-G estrone-3-glucuronide and P-3-G (progestrone-3-glucuronide)), polynucleotides, steroids, lipoproteins, drugs, bacterial or viral antigens, such as Streptococcus, Neisseria and Chlamydia, lymphokines, cytokines, and the like. A number of suitable analytes are described in U.S. Patent No. 5,686,315, which is incorporated herein by reference. Although examples are provided for the determination of fetal fibronectin in cervicovaginal samples, the systems and methods provided herein are not limited to the detection and measurement of fetal fibronectin, but apply to any biochemical test, particularly those for which test strips can be developed or for which test strips are known.

Measurement of Fetal Fibronectin

In an exemplary embodiment, the system is used for diagnosing or predicting conditions such as pregnancy, including ectopic pregnancy, pre-eclampsia, preterm labor or imminent delivery, fetal membrane rupture or infections of concern during pregnancy that may compromise the pregnancy or

harm the newborn, including, for example, ascending genital infections such as chlamydia or herpes simplex virus. Fetal fibronectin is a fetal restricted antigen found in placenta, amniotic fluid and fetal connective tissue. Since fetal fibronectin is strictly associated with pregnancy, determination of the presence of fetal fibronectin in a cervicovaginal sample is a highly reliable early indication of pregnancy. Also, the absence of a fetal restricted antigen in a cervicovaginal sample during the first 20 weeks of pregnancy is an indicator of ectopic pregnancy. Ectopic pregnancies, which are a major cause of mortality and mortality for women, are not readily distinguished from normal pregnancies using standard pregnancy determination methods and tests. Determination of impending preterm births is critical for increasing neonatal survival of preterm infants. The presence of fetal fibronectin (fFN) in cervicovaginal secretion samples in patients after week 12 of pregnancy has been shown to be associated with a risk of impending delivery, including spontaneous abortions (12-20 weeks), preterm delivery (20-37 weeks), term (37-40 weeks) and post-date delivery (after 40 weeks), in pregnant women. In addition, the presence of fetal fibronectin in a cervicovaginal sample provides a method for determining increased risk of labor and fetal membrane rupture after week 20 of pregnancy. Detection of rupture of the amniotic membrane is important in distinguishing true and false labor, and when the rupture is small and the volume of amniotic liquid escaping is small, the rupture is often undetected. The methods and systems herein provide a means to reliably assess the risk for any of these conditions. An immunoassay procedure for detecting fetal fibronectin is described in EXAMPLE 2.

Test Strip for Measuring fFN and Cellular Fibronectin

Methods for measuring fetal fibronectin and cellular fibronectin levels in cervicovaginal samples are known (see, U.S. Patent Nos. 4,919,889, 5,079,171, 5,096,830, 5,185,270, 5,223,440, 5,236,846, 5,281,522, and 5,468,619), and diagnostic tests for various pregnancy-related disorders are available (see, e.g., U.S. Patent Nos. 5,096,830 and 5,079,171) in which levels of estriol in saliva are correlated with risks of preterm delivery; International PCT application No. WO 93/09438 and U.S. Patent No. 5,516,702, which describes

the use of cytokines as indicators of risk of preterm delivery and of membrane rupture). Other tests that can be incorporated into systems provided herein include tests in which the level of insulin-like growth factor binding protein 1 (IGFBP-1) in a vaginal secretion sample, resulting from the rupture of fetal membranes, is used to detect rupture of fetal membranes (see, International PCT application No. WO 92/12426 and U.S. Patent No. 5,554,504; and see also International PCT application No. WO 94/17405 or EP A 0 680 607, which describes the use the levels IGFBP-1 in cervicovaginal samples from women determined to be at risk of preterm delivery as a means for determining the risk of impending preterm delivery). These methods can be adapted for use with the immunoassay test strips and devices described herein. In particular, an immunoassay test strip for measuring fFN in cervicovaginal samples is provided.

Antibodies for Fetal Fibronectin

An antibody that will bind the analyte of interest is conjugated to a detectable label. The selected antibody will depend upon the particular test used. Such antibodies are readily available.

In an exemplary embodiment, where fetal fibronectin is to be detected, a mouse monoclonal anti-fFN antibody (deposited at the American Type Culture Collection as accession number ATCC HB 9018; see U.S. Patent No. 4,894,326; see, also U.S. Patent Nos. 5,281,522 and 5,243,029), conjugated to latex particles containing a blue dye may be used. In another embodiment, embodiment, a goat polyclonal antibody to human fibronectin is conjugated to a colloidal gold label.

In the embodiment, an antibody that binds the labeled antibody conjugate that is not complexed with fetal fibronectin is used as a control antibody. For example, where the labeled conjugate includes a monoclonal anti-fetal fibronectin antibody, a polyclonal goat anti-mouse IgG antibody is used.

The antibodies may be raised and purified using methods known to those of skill in the art or obtained from publicly available sources. For example, monoclonal antibody FDC-6 (deposited at the American Type Culture Collection as accession number ATCC HB 9018; see U.S. Patent No. 4,894,326; see, also, Matsuura et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:6517-6521; see,

also, U.S. Patent Nos. 4,919,889, 5,096,830, 5,185,270, 5,223,440, 5,236,846, 5,281,522, 5,468,619 and 5,516,702), which is raised against whole molecule onco-fetal fibronectin from a tumor cell line, may be used.

Fetal Fibronectin Assay Procedure

The fetal fibronectin assay is provided as exemplary of assays and design thereof for use in the systems provided herein.

In conducting the assay, a cervicvaginal patient sample is obtained. The sample may include fluid and particulate solids, and, thus, can be filtered prior to application to the assay test strip. The sample may be removed from the patient using a swab having a fibrous tip, an aspirator, suction or lavage device, syringe, or any other known method of removing a bodily sample, including passive methods for collecting urine or saliva. In particular, the sample may be extracted into a buffer solution, and optionally heated, for example, at 37°C and filtered. In the embodiment, where fetal fibronectin is to be detected in a sample, the sample is obtained from in the vicinity of the posterior fornix, the ectocervix or external cervical os using a swab having a dacron or other fibrous tip.

A volume of the test sample is then delivered to the test strip (FIG. 1) using any known means for transporting a biological sample, for example, a standard plastic pipet. Any analyte in the sample binds to the labeled antibody and the resulting complex migrates along the test strip. Alternatively, the sample may be pre-mixed with the labeled conjugate prior to applying the mixture to the test strip. When the labeled antibody-analyte complex encounters a detection zone of the test strip, the immobilized antibody therein binds the complex to form a sandwich complex, thereby forming a colored stripe.

Any unbound latex-conjugated antibody continues to migrate into a control zone where it is captured by a second immobilized antibody or other agent capable of binding the conjugate, and thereby forms a second colored stripe due to the accumulation of the dye-containing latex beads.

The results of the assay are assessed using the reader and software

The color intensity produced is read, in accordance with the present embodiment, using a reader device, for example, a reflectance reader, adapted to read the test strip. The intensity of the color reflected by the colored label in the test region (or detection zone) of the test strip is directly proportional to the amount of analyte present in the sample being tested. In other words, a darker colored line in the test region indicates a greater amount of analyte, whereas a lighter colored line in the test region indicates a smaller amount of analyte. In accordance with the present embodiment, the color intensity produced, i.e., the darkness or lightness of the colored line, is read using a reader device, for exampie, a reflectance reader, adapted to read the test strip. A reflectance measurement obtained by the reader device is, in accordance with the present embodiment, correlated to the presence and/or quantity of analyte present in the sample as described hereinbelow. The reader takes a plurality of readings along the strip, and obtains data that are used to generate results that are an indication of the presence and/or quantity of analyte present in the sample as described hereinbelow. The system also correlates such data with the presence of a disorder, condition or risk thereof.

Optionally, in addition to reading the test strip, the reader may be adapted to read a symbology, such as a bar code, which is present on the test strip or housing and encodes information relating to the test strip device and/or test result and/or patient, and/or reagent or other desired information. Typically the associated information is stored in a remote computer database, but can be manually stored. In other embodiments, the symbology can be imprinted when the device is used and the information encoded therein.

Referring to FIG. 6, an exemplary embodiment of the reader device **600** is shown with an immunoassay device **200**, as shown in FIG. 2A, inserted into a cassette slot **602** therein. The cassette slot **602** is adapted to receive the immunoassay device **200**, and a reader head assembly (not shown) supported within the reader device **600** is adapted to read the immunoassay test strip, and optionally a symbology, exemplified as a bar code, on with the immunoassay device. Such reading is performed by scanning a reader head (not shown) across the device, including a test window **214** in the immunoassay device **200**

and a symbology, such as the exemplified bar code **216**, if present, and in the process directing light onto the bar code and/or a test portion and a control portion of the immunoassay test strip. An amount of such light reflected back from the bar code and/or the test portion and control portion of the immunoassay test strip is measured as the reader head scans across the device.

Also shown are a data entry keypad **604**, including ten digit keys (also labeled with letters of the alphabet, such as is commonly the case on telephone keypads), a delete key, a space key, an escape key, a print key, enter key, and up, down, left and right arrow keys, additional characters such as , or . or /, and any others desired by the user. The data entry keypad **604** can be used by an operator of the reader device **600** to input identification information, to enter control test parameters, to initiate and terminate testing, and the like. A processing unit (not shown) housed within the reader device **600** is responsive to the keypad and performs data analysis functions, as described hereinbelow, in accordance with modifications made to a processor in the processing unit by an appropriate software subsystem.

Also shown in FIG. 6 is a liquid crystal display screen **606**. The liquid crystal display screen **606** receives output data from the processing unit and displays it to an operator of the reader device **600**, including displaying results of tests, error messages, instructions, troubleshooting information, and the like.

Referring next to FIG. 7, a perspective view of a lower housing **702** of one embodiment of an immunoassay reader device **600** of FIG. 6 is shown with a reader head assembly **704** located therein and the immunoassay device **200** inserted into the cassette slot **602** at a front edge of the lower housing **702**. The cassette slot **602** located at the front edge of the lower housing **702** provides an aperture through which the immunoassay device **200** is inserted into and guided into the reader device **600** in order measure light reflected from an immunoassay test strip. In some embodiments of the reader, the reader is adapted to additionally read a symbology, such as a bar code, imprinted, engraved on or otherwise affixed to the test strip or device.

When the immunoassay device **200** is inserted into the cassette slot **602** of the lower housing, a reader head **706** on the reader head assembly **704** is

positioned directly above the device **200**, such that the longitudinal (or major) axes of optical fibers within the reader head **706** are normal to a surface of the device, including the test strip and optionally a symbology that is imprinted, engraved or other wise affixed on the device.

Alternatively, the reader head **706** may be fixed, at least rotationally, and the immunoassay device **200** may be moved into position after insertion into the cassette slot **602**, such that the longitudinal (or major) axes of optical fibers within the reader head **706** are normal to a surface of the device to be read by the reader.

Referring next to FIG. 8, a top view is shown of the lower housing **702**, the immunoassay device **200**, the cassette slot **602**, and a stepper motor **802**. As can be seen, after insertion into the lower housing **702**, the immunoassay device **200** is positioned in alignment with the stepper motor **802**, which is part of the reader head assembly. The stepper motor is used to scan the reader head **706** across the symbology, such as the exemplified bar code **216** and/or test window **214** of the immunoassay device **200**.

One embodiment of the reader device is shown in FIG. 9. Shown are the lower housing **702**, the immunoassay device **200**, the stepper motor **802**, an actuator wheel **902**, the reader head **706**, and linkages for moving the reader head **706** parallel to a major axis of the immunoassay device **200** in order to scan the reader head **706** across the symbology (bar code) **216** and/or test window **214** of the immunoassay device **200**.

To read the immunoassay test strip, the reader head is brought within a uniform distance of about 0.010 inches from the test strip. When the immunoassay device **200** is slid into the cassette slot **602**, the actuator wheel **902** and an actuator spring (not shown) work together to bring the reader head **706** down to within about 0.010 inches of the immunoassay test strip within the housing **202** of the immunoassay device **200**. In order to move the reader head **706** into position within 0.010 inches of the immunoassay test strip, the reader head **706** is pivoted along with a portion of the reader head assembly. Prior to being brought into position within 0.010 inches of the immunoassay test strip, while the immunoassay device **200** is being inserted into or removed

from the immunoassay reader device **600**, the reader head **706** assumes a retracted position, i.e., raised position, so that the immunoassay device **200** can be inserted into or removed from the immunoassay reader device **600** without crashing the reader head **706** into the immunoassay device **200**.

When the immunoassay device **200** is inserted into the cassette slot **602**, it contacts the actuator wheel **902** and causes a carriage assembly of the reader head assembly to be brought down from the retracted position so that the reader head **706** is within 0.010 inches of the immunoassay test strip.

Insertion of the immunoassay device **200** causes the actuator wheel to pop-up by applying pressure to the actuator spring, bringing the carriage assembly down from the retracted position.

The immunoassay device **200** is pushed into the cassette slot **602** until it meets a stop. Once inserted, the immunoassay device **200**, the actuator wheel **902**, and the actuator spring remain fixed in position, while the reader head **706** is stepped across the test window **214** of the immunoassay device **200** by the stepper motor **802**. In other words, only the reader head **706** moves during the scanning of the immunoassay test strip.

Alternatively, the immunoassay device **200** is pushed into the cassette slot **602** until it meets the stop. Once inserted the immunoassay device **200** may be rotated up to within 0.010 inches of the reader head **706** by gently lifting the immunoassay device **200**. By gently lifting the immunoassay device **200**, a base of the reader head assembly is pivoted up toward the carriage assembly and the reader head **706**, positioning the immunoassay test strip within 0.010 inches of the reader head **706**. Then the reader head **706** is then stepped across the test window **214** of the immunoassay test strip by the stepper motor **802**. In other words, in accordance with this alternative, only the reader head **706** moves during the scanning of the immunoassay test strip and, the reader head **706** moves only during the scanning of the immunoassay test strip.

Prior to insertion of the immunoassay device **200** into the cassette slot **602**, and prior to scanning, the reader head **706** is positioned at a point that would place it approximately half way across (in the middle of) the test window

214 of the immunoassay device 200. After insertion of the device 200 into the reader 600, when an operator depresses a scan key on the key pad (see FIG. 6), the reader head 706 is moved from this position toward the stepper motor 802 until a microswitch is activated. Once the microswitch is activated, the reader head 706 is said to be in a "home" position from which scanning of the test strip commences. Once scanning commences, the reader head 706 advances from the home position across the test window 214. Thus, the reader head 706 scans in a direction moving away from the stepper motor toward the cassette slot 602 or to the left as depicted in FIG. 9. Total travel of the reader head 706 during scanning of the immunoassay test strip is 0.452 inches, which is achieved in 0.002 inch steps, which are 226 in number. One set of readings is taken per step, with each set of readings including a dark reading, a first light reading and a second light reading.

Referring next to FIG. 10, the reader head assembly 1000 is shown. Shown are the actuator spring 1002, the actuator 1004, the base 1006, the stepper motor 802, the actuator wheel 902, a rotor arm 1008, and the reader head 706. Also shown is a pivot point 1010 on which the carriage assembly 1012, including the reader head 706, stepper motor 802, actuator wheel 902, actuator spring, 1002, and rotor arm 1008 pivot to assume a raised position for insertion and removal of the immunoassay device 200 from the reader device 600 and to assume a lowered position for scanning the reader head 706 across the test window 214 of the immunoassay device 200.

Referring next to FIG. 11, shown is a side view of the reader head 706 of the reader head assembly of FIG. 10. Shown is a first light emitting diode (LED) 1102, a second light emitting diode (LED) 1104, a photodetector 1106, a reader head aperture 1108, and mounting holes 1110.

Referring next to FIG. 12 a reverse angle side view is shown of the reader head assembly 1000 of FIG. 10. Shown are the stepper motor 802, the base 1006, mounting holes 1202, and a mounting bracket 1204 on which the reader head 706 is mounted.

Referring next to FIG. 13 a reverse angle side view of the reader head **706** of FIG. 11 is shown. Seen are the mounting holes **1110**, and the reader head aperture **1108**.

Referring next to FIG. 14, shown is a side view of the reader head assembly **1000** of FIG. 10 having assumed a retracted position. Shown are the actuator spring **1002**, the actuator arm **1004**, the stepper motor **802**, the reader head **706**, the reader head supporting bracket **1204**, the pivot **1010** on which such elements rotate, and a base **1006** relative to which such elements rotate.

As can be seen, the actuator arm **1004**, the actuator spring **1002**, the stepper motor **802**, the reader head **706**, the reader head mounting bracket **1204**, and mechanisms used for supporting and scanning the reader head **706** are designed so that the test strip **214** in the device **200** is positioned within 0.010 inches of the aperture **1108** of the reader head. Any design suitable to effect such can be employed with the present embodiment.

In the example illustrated, the actuator arm **1004**, the actuator spring **1002**, the stepper motor **802**, the reader head **706**, the reader head mounting bracket **1204**, and the mechanisms used for supporting and scanning the reader head **706** are shown rotated on the pivot **1010** such as would be the case, in accordance with the variation shown, when the immunoassay device **200** has been removed from the reader device **600** and/or as the immunoassay device **200** is being inserted into or removed from the reader device **600**.

Referring next to FIG. 15, a side view is shown of the reader head **706** of FIG. 11. Shown are the aperture **1108** and the first light emitting diode **1102**.

Referring next to FIG. 16, an end view is shown of the reader head assembly **1000** of FIG. 10. Shown is the stepper motor **802**, the base **1006**, and the actuator arm **1004**.

In alternative embodiments, the reader is adapted to read a symbology, such as a bar code. An exemplary embodiment of a reader so-adapted is shown in FIG. 24. In this embodiment, when the device cassette **2402** is inserted into the reader, it sits on a spring stage **2404**. Prior to insertion of the device **300**

(as shown in FIG. 2B) into the cassette slot **602**, and prior to scanning, the reader head **2406** is positioned at a point that would place it approximately 0.125 inches from the forward edge of the device as it is inserted into the reader.

As shown in FIG. 2B, the device includes guide ridges **318** on either side of the bar code along the outer edges of the upper surface of the device. The reader head **2406** is moved by the shaft **2408** of the stepper motor **2414** and scans in a direction moving away from the stepper motor toward the cassette slot **2412**. As the reader head **2406** moves along the device above the bar code **2410**, the guide ridges **2412** contact the reader head assembly and act to compress the spring stage **2404** by 3° in order maintain the reader head **2406** at a distance of 0.010 inches above the bar code **2410** as the bar code **2410** is read. After the bar code **2410** is read, the reader head assembly **2406** moves off the guide ridges **2412**, the spring stage **2404** returns to a level position (0°), and the reader head **2406** is repositioned at a distance of 0.010 inches in order to read the test strip. When reading a symbology, such as a bar code, the reader is moved in steps of approximately between 0.002-0.008 inches at a scan resolution of approximately 125-500 steps per inch, preferably about 250 steps per inch. One set of readings is taken per step, with each set of readings including a dark reading, a first light reading and a second light reading.

Regardless of whether one of these alternatives is used, or whether any of numerous variations thereof or any of numerous other possible embodiments well within the abilities of the skilled artisan to easily produce is used in order to position the reader head within a prescribed distance, e.g., 0.010 inches, of the test strip symbology, such as a bar code, a suitable mechanism is preferably employed to effect such positioning.

Referring next to FIG. 17, shown is a side view partially in cross-section of an exemplary embodiment of the reader head. Shown are a first light emitting diode **1102**, a second light emitting diode **1104** and a photodetector **1106**. Also shown is an aperture **1108** and the mounting holes **1110**. Shown coupled between each of the LED's **1102**, **1104** and the aperture **1108** are first and second fiberoptic bundles **1702**, **1704**. Similarly, a third fiberoptic bundle

1706 is shown coupled between the aperture 1108 and the photodetector 1106. The first and second fiberoptic bundles 1702, 1704 conduct light from the first and second LED's 1102, 1104, respectively, to the aperture 1108. The third fiberoptic bundle 1706 conducts light from the aperture 1108 to the photodetector 1106. In response to such light, the photodetector generates a reflection signal, e.g., a voltage indicative of an amount of reflected light. The electrical signal can be then processed and converted to a digital signal by using any method commonly known to those of skill in the art.

Referring next to FIG. 18, shown is a closeup partial cross-sectional view of the aperture 1108 of FIG. 17. Also shown are individual fiberoptic fibers 1802, 1804, 1806 of the fiberoptic bundles 1702, 1704, 1706 of FIG. 17, positioned within the aperture 1006 so as to transmit light 1808 from the aperture 1108 onto the symbology (exemplified bar code) and/or test strip 100 and to receive reflected light 1808 from the bar code and/or test strip 100 entering the aperture 1108. (The transmitted and reflected light 1808 is represented with an arrow.) As can be seen, a gap 1810 between the aperture 1808 and the bar code and/or test strip 100 is present. The gap 1810 preferably has a width of approximately 0.010 inches, which is maintained as the reader head 706 is scanned across the bar code and/or test window 214 of the test strip 100.

Referring next to FIG. 19, a bottom view is shown of individual fiberoptic fiber ends 1902, 1904, 1906 positioned in the aperture 1108 of the reader head 706 of FIG. 10 so as to maximize the distribution of light emitted from individual fiberoptic fibers (fiberoptic conductors), and furthermore to maximize the uniformity of light received into individual fiberoptic conductors. Indicated using diagonal cross-hatching angled from lower left to upper right are individual fiberoptic conductor ends 1902 of the first fiberoptic bundle 1702. These individual fiberoptic conductor ends 1902 carry light emitted from the first light emitting diode from the first fiberoptic bundle through the aperture 1108 of the reader head 706. Similarly, indicated with cross-hatching from an upper left to lower right are fiberoptic conductor ends 1904 of the second fiberoptic bundle 1704. These individual fiberoptic conductor ends carry light emitted from the

second light emitting diode to the aperture **1108** of the reader head **706**. Without cross-hatching are shown individual fiberoptic conductor ends **1906** of the third fiberoptic bundle **1706**. The third fiberoptic bundle **1706** carries light entering the aperture **1108** to the photodetector.

By employing the particularly advantageous arrangement of the fiberoptic conductor ends **1902**, **1904**, **1906** at the aperture **1108**, uniform distribution emissions and light reception is achieved. Such arrangement is said to be a "sigmoidal" (S-like or serpentine) arrangement or a "sigmoidal" distribution. It is an important feature of the present embodiment that the fiberoptic fibers in each of the three groups are arranged along with fiberoptic fibers from the remaining groups in a sigmoidal-like (or "S"-like) pattern with three columns of thirteen fiberoptic fibers each. An arrangement that achieves this feature is intended herein.

In order to achieve the sigmoidal arrangement of fiberoptic conductor ends shown, 39 fiberoptic conductors are positioned within the aperture **1108**. Next, a clamp assembly made up of a "U"-shaped channel, and an "I"-shaped clamp positioned at the open side of the "U" is employed. The fiberoptic conductors, portions of which protrude from the aperture **1108** are placed between the "U"-shaped channel and the "I"-shaped clamp and a compressive force is applied thereto by the "I"-shaped clamp, holding the protruding portions of the fiberoptic conductors firmly in position. A resin is then poured into the reader head **706** so as to become interposed between and around the fiberoptic conductors at the aperture **1108**. Once the resin is set, the clamp assembly is removed, and the protruding portions of the fiberoptic conductors are trimmed back flush with the aperture **1108**, so as to define a planar surface of fiberoptic conductor ends **1902**, **1904**, **1906** at the aperture **1108**. This planar surface is held parallel to a plane at an upper surface of the immunoassay test strip **100** during scanning of the immunoassay test strip.

Advantageously, by creating this planar surface of fiberoptic conductor ends **1902**, **1904**, **1906**, the associated fiberoptic conductors of which all of which have longitudinal axes that are substantially parallel to one another and normal to the plane defined by the fiberoptic conductor ends **1902**, **1904**,

1906. As a result, very efficient transfer of light to and from the fiberoptic conductor ends 1902, 1904, 1906 is achieved.

Once the fiberoptic conductor ends 1902, 1904, 1906 are set in the resin, and trimmed, as described above, in individual fiberoptic conductors are tested by projecting light individually through the fiberoptic conductors toward the fiberoptic conductor ends 1902, 1904, 1906, to locate the fiberoptic conductor end associated with the particular fiberoptic conductor being tested. This determination is made by observing which of the fiberoptic conductor ends 1902, 1904, 1906 "lights up" when light is transmitted down the particular fiberoptic conductor. As fiberoptic conductors associated with the fiberoptic conductor ends 1902, 1904, 1906 are identified, the fiberoptic conductors are assigned to one of the first, second, and third fiberoptic bundles, so as to achieve, for example, the sigmoidal distribution of fiberoptic conductor ends 1902, 1904, 1906 illustrated in FIG. 19.

Advantageously, by effecting the sigmoidal distribution of fiberoptic conductor ends 1902, 1904, 1906 associated with fiberoptic conductors of each of the first, second, and third fiberoptic bundles, a uniform distribution of light emitted from the aperture 1108, and a uniform distribution of light reflected back to the aperture 1108 is achieved.

Referring next to FIG. 20, a cross-sectional view of a first fiberoptic bundle 1702 is shown with individual fiberoptic elements 2002 selected to effect the sigmoidal distribution of FIG. 19. As can be seen, 13 individual fiberoptic elements are present in the fiberoptic bundle 1702, which is the same number of fiberoptic conductor ends 1902, 1904, 1906 depicted in FIG. 19 for each of the three fiberoptic bundles 1702, 1704, 1706. The fiberoptic bundle 1702 shown in FIG. 17 carries the light from the first light emitting diode to the aperture 1108 of the reader head 706. Cross-sectional views of the second and third fiberoptic bundles are similar to that shown in FIG. 20.

Referring next to FIGs 21, 22 and 23, three schematic views are shown illustrating a process for reading test results from immunoassay test strip 100 with the control region 2102 and the detection region 2104 depicted thereon. In the example shown, blue latex particles are detected in the test region and

the control region on a nitrocellulose support. Also depicted are the aperture **1108** of the reader head **706** in a dark reading mode (FIG. 21), a first light reading mode (FIG. 22) and a second light reading mode (FIG. 23).

The reader head assembly (described above) includes the first light-emitting diode (which in the present example is a blue LED), the second LED (which in the present example is an amber LED), a silicon photodiode detector, and fiberoptic fibers arranged with fiberoptic conductor ends **1902**, **1904**, **1906** in the sigmoidal distribution in the aperture **1108** (e.g., 0.002 inches wide), which is located at the bottom (or tip) of the reader head **706** at a point nearest the immunoassay test strip, when the immunoassay device is inserted into the immunoassay reader device. It is understood that the selection of LEDs will be dependent upon the signal produced in the test; all detectable electromagnetic wavelengths, preferably visible light, are contemplated herein. Fluorescence and other such labeling means are also contemplated herein.

The blue LED and the amber LED emit light of specified wavelengths (λ_1 and λ_2, respectively). It should be understood that any suitable wavelengths may be selected. Such selection is dependent on the particular assay with which the immunoassay reader device is being employed. The wavelengths selected are selected so as to allow removal of effects of the background of the immunoassay test strip or symbology, e.g., bar code, from the reflectance readings, and to optimize a reading of a reduction in reflectance associated with accumulated label at the reaction regions of the immunoassay test strip.

In an exemplary embodiment, where blue latex particles are detected on a nitrocellulose support, light having a wavelength of 430 nm (blue) is emitted from the first light emitting diode (LED_1), i.e., the blue LED, into the first fiberoptic bundle **1702**. The same wavelengths can be used to read a symbology, such as a bar code, associated with the assay device. The first fiberoptic bundle **1702** transmits blue light to the aperture **1108** in the reader head **706** where it is emitted at an orientation normal to a plane at the upper surface of the symbology (exemplified bar code) or test strip. A second light emitting diode (LED_2), i.e., the amber LED, emits light with a wavelength of 595 nm (amber) into a second fiberoptic bundle **1704**. The second fiberoptic bundle

1704 transmits the amber light to the aperture in the reader head 706 where it is emitted at an orientation normal to the plane at the upper surface of the bar code or test strip.

At the aperture, individual fiberoptic conductor ends 1902, 1904 of the first and second fiberoptic bundles 1702, 1704, along with individual fiberoptic conductor ends 1906 from the third fiberoptic bundle 1706 are arranged in three groups of thirteen optical fibers each: the first group from the first fiberoptic bundle 1702, which transmits light emitted by the blue LED to the aperture 1108; the second group from the second fiberoptic bundle 1704, which transmits light emitted by the amber LED to the aperture 1108; and the third group, which transmits reflected light received at the aperture 1108 through the third fiberoptic bundle 1706 to the photodetector. The thirty-nine fibers (thirteen in each of three groups) each include respective fiberoptic conductor ends 1902, 1904, 1906 arranged in the sigmoidal distribution (or pattern) (see FIG. 19) at the aperture 1108 such that the fiberoptic conductor ends 1902, 1904, 1906 are co-planar at the aperture and in the plane parallel to the plane at the upper surface of the bar code or test strip, when the reader head 706 is positioned to take measurements from the bar code or test strip 100.

At the fiberoptic conductor ends 1902, 1904, 1906, each fiberoptic fiber (or conductor) has a longitudinal axis that is normal to the plane at the upper surface of the bar code or test strip. As a result, light emitted from the fiberoptic conductor ends 1902, 1904, 1906 is directed in a direction substantially normal to this surface plane. The fiberoptic fibers in each of the three groups are arranged along with fiberoptic fibers from the remaining groups in a sigmoidal (or "S"-like) pattern with three columns of thirteen fibers each.

When the immunoassay device is inserted into the cassette slot at the front of the immunoassay reader device 600, the reader head 706 is positioned directly over the bar code or test opening of the assay device such that the longitudinal axes of the optical fibers at their ends 1902, 1904, 1906 at the aperture, are normal to a plane at the surface of the immunoassay test strip and the ends 1902, 1904, 1906 of the fibers at a distance of about 0.010 inches.

Light from the first LED and the second LED is transmitted by the fibers onto the bar code or assay test strip at an angle normal to the upper surface of the immunoassay device, and light is reflected normally back from the strip to the ends **1902**, **1904**, **1906**. This reflected light is transmitted by the fibers of the third fiberoptic bundle to the photodetector.

The reader head **706** takes three separate reflectance readings (FIGs. 21, 22 and 23, respectively) from each position at which it reads of the immunoassay test strip. Such measurements are made by reading an output of the photodetector (which is a voltage) while controlling the first and second LED's.

The first reading is used to determine an amount of ambient (or background) light leaking into the immunoassay device (e.g., light leaking through the cassette slot entrance, or light reflected/transmitted into the reader through the housing of the immunoassay device, which may be, for example, white plastic. The first reading is a "dark" reading taken with the blue LED and the amber LED both turned off. This dark reading (which is a voltage at the photodetector) is digitized in a conventional manner using an analog to digital converter, and may be subtracted by the processing unit from other "light" readings made in response to blue LED illumination and amber LED illumination so as to correct for this light leakage.

The second reading, used to determine levels of light reflections associated with the background of the bar code or the assay test strip itself, is taken with the blue LED pulsed on and the amber LED turned off.

The third reading, used to detect the bar code or the presence of the label on the assay test strip is taken with the amber LED pulsed on and the blue LED turned off.

A control circuit (including the processing unit, which includes a processor, such as a microprocessor) receives the digitized output of the photodetector for all three readings, controls the on and off operation of the blue LED and the amber LED, controls when photodetection readings are taken, and controls the position of the reader head **706** by controlling the stepper motor. A memory circuit stores raw and/or processed data (i.e., readings from

the photodetector). The data may also be displayed to the operator via the LCD display of the immunoassay reader device **600**.

After being positioned above the housing, the reader head **706** is moved (scanned) across the bar code and/or test strip by the stepper motor under the control of the control circuit to allow the reader head **706** to scan the exposed surface of the bar code and/or assay test strip (including the detection and control zones through the test window **214** in the immunoassay device). As stated above, in a preferred embodiment, the distance between reader head **706** and the bar code or assay test strip **200** is approximately 0.010". The precise distances for any system may be empirically determined and will vary between systems and even among similar systems.

The reader head **706** is slidably connected to a rail (e.g., guide rods), and is coupled to a worm or screw gear driven by the stepper motor.

Under the control of the control circuit, the stepper motor drives the reader head **706** along the rail in small steps. At each step, the control circuit takes the three readings described above ("dark", blue LED illuminated, amber LED illuminated). The control circuit moves the reader head **706** such that the fiberoptic conductor ends **1902**, **1904**, **1906** pass directly above and normal to the exposed surface of the bar code and/or test strip in a sequence of small steps. As explained above, during each step a sequence of "dark", blue LED and amber LED readings are taken and recorded.

The raw data read from the photodetector is then processed by the control circuit to discern the symbology, such as a bar code pattern, in order to provide information regarding the assay device and/or test run and/or reagents, and/or patient and/or to read the test strip to determine the presence or concentration of analyte in the sample.

In a preferred embodiment, when reading the test strip, since the detection and control latex stripes are each about 0.020" wide, and since each step of the sensing head is about 0.002" long, there will be about 10 steps within each stripe, i.e., within the test region and the control region. Thus, there will be 10 sets of three readings (i.e., dark, blue LED and amber LED) at the test region and 10 sets of three readings (i.e., dark, blue LED and amber

LED) at the control region. The remainder of the reading sets will not be made over either the test region or the control region.

In a preferred embodiment, when the assay device is inserted into the cassette slot of the reader device **600**, the reader head **706** is positioned over the bar code or test strip, and the control circuit then moves the head to an initial (or home) position. The control circuit moves (scans) the head across the exposed surface of the bar code or test strip, including the test region and the control region of the strip, in small increments. At each step, the control circuit takes the first reading (FIG. 21) of the photodetector output with the blue LED and the amber LED, both off, takes the second reading (FIG. 22) with the blue LED pulsed on and the amber LED off, and takes a third reading (FIG. 23) with the blue LED off and the amber LED pulsed on. The control circuit then steps the reader head **706** by controlling the stepper motor and repeats the three readings at its new location. For the test strip, this process is repeated for each of 226 steps (0.452" at 0.002"/step) until the surface of the assay test strip is read. Where a bar code is read, the length of the bar code is typically approximately 1 inch in length, and a step size of approximately 0.002-0.008 inches are used; thus between approximately 125-500 steps are performed.

The raw reflectance data is then analyzed by the control circuit in accordance with appropriate software control to identify the symbology, such as a bar code or determine the presence or concentration of the analyte in the sample. Where the reader is used to read a bar code associated with the test device, the data collected from the bar code are transformed into integrated peak information and analyzed as alphanumeric characters to provide information about the assay device and/or test run. Where the reader is used to detect an analyte, the data collected from the test strip are compared to a threshold or reference reflectance value to determine the presence or concentration of the analyte. The output can be displayed via an operator interface, or can be output to another computer or apparatus.

Data Analysis and Decision Support Systems

The systems herein include software for data analysis. Data analysis includes any algorithms or methodology for obtaining diagnostically relevant

information from the raw data. Simple algorithms as well as decision-support systems, particularly neural networks are contemplated herein.

In particular embodiments, the data analysis methodology includes, some or all of the following steps: (1) optionally correcting the reflectance readings to correct for light leakage; (2) reducing the raw reflectance data by using a ratiometric formula; (3) generating an image of the test data by plotting the reduced data; (4) expressing this image as a polynomial mathematical function, for example, by using a combination of a flat or parabolic image to represent the baseline and two gaussian curves to represent the peaks; (5) using a curve-fitting algorithm to generate parameters to define the image; (6) optimizing the reconstruction of the image and producing a fitted image; (7) comparing the scanned image and fitted image by solving the linear regression through the curves; (8) validating the parameters obtained from the curve-fitting and the peak heights obtained; and (9) classifying the validated result as positive or negative by comparing peak heights of a clinical sample to reference samples. The method may further include: (10) using the test result with other patient information in a decision-support system to generate a medical diagnosis or risk assessment.

In alternative embodiments, the parameters used to define the image, as in (5) above, and to classify the sample, as in (9) above, can be generated using trained neural networks.

Data Reduction

In an exemplary embodiment, the raw reflectance data obtained from the instrument are stored as an array of points containing a number of rows (n) corresponding to the number of points at which readings were taken along the test strip, and a number of columns (m) corresponding to the reflectance readings taken at each point, including background or dark readings and readings at different wavelengths. If necessary, the reflectance readings are processed by first subtracting the dark reading taken at the corresponding step to correct for light leakage, which typically is negligible. The corrected reflectance readings are then input into a ratiometric algorithm, which removes noise from the membrane and normalizes data between test strips:

$f(y) = [(R_{\lambda 1}/R_{max/\lambda 1} * R_{max/\lambda 2}/R_{\lambda 2})]$.

The algorithm is based upon the ratio of readings at the different wavelengths and calculates a reduced data set (1 x n), which is used to generate a curve from the original reflectance data. In processing the data, a new column of reduced data is generated by using the ratiometric formula.

When reading an assay test strip, as described above, the size of the matrix is 4 x 226, where 4 is the number of columns of data collected and 226 is the number of steps, or readings, taken along the test strip. The first column contains information about the location on the test strip from which the data are obtained; the second column is the reflectance in the absence of illumination by the instrument (dark reading); the third column is the reflectance when the test strip is illuminated at the first wavelength (e.g. 430 nm); and the fourth column is the reflectance when the test strip is illuminated at the second wavelength (e.g. 595 nm). The information in the second column is usually zero, unless a light breach has occurred with the instrument. The reflectance values in the third and fourth column are preferably in the 3,000-24,000 range.

Where a bar code is read, between approximately 125-500 steps are performed in reading the bar code, therefore, the matrix size would be between 4 x 125 and 4 x 500.

In the preferred embodiment described herein, the ratiometric formula would read as follows:

$f(y) = [(R_{430nm}/R_{max/430nm} * R_{max/595nm}/R_{595nm})] - 1$.

The algorithm calculates a reflectance ratio for each step, generating a fifth column of data. The information contained in the first, third and fourth columns can be converted into an image by plotting the first column (x-value) against the fifth column (y-value). Thus, the original data array has been converted to a 2-dimensional image, or an array of the size 1 x 226. The reflectance ratio is then plotted as a function of each step. In reading an assay test strip, as described above, the result is a two-peak graph with the peaks occurring at the two stripes, corresponding to the detection and control zones. The reflectance data may then be further processed to obtain an accurate determination of analyte concentration in the patient sample.

Where a bar code is read, a graph is produced that corresponds to the reflectance pattern of the bar code. Pattern matching is then performed using any of a number of methods commonly known to those of skill in the art in order to identify the bar code and associate it with the particular assay run.

Generating and Validating Images

The image created by a plot of the data obtained from reading an assay test strip, as described above, has three basic components: a baseline or background that is either flat or parabolic; a peak corresponding to the detection zone that is gaussian; and another peak corresponding to a control zone that is also gaussian.

The parabolic component can be defined using 3 variables:

$$f(y) = Ax^2 + Bx + C.$$

Each of the gaussian curves can be defined using 3 variables:

$$f(y) = \text{Area} * [\exp^{-(x-\mu)(x-\mu)/2\sigma*\sigma}]/(\sigma(2\pi)^{1/2})$$

where Area = area contained within the gaussian;
μ = x-value of center position; and
σ = width.

A second plot can be generated from the three component curves, using 9 variables. This process is accomplished using a curve-fit algorithm. Such algorithms are well known to those of skill in the art, and any such algorithm may be used. Alternatively, the 9 parameters may be obtained using neural networks, as described below. From the parameters generated from the curve-fit function, a showfit function is used to generate an image from the fitted data. For example, in the preferred embodiment, a showfit function is used to generate a 1 x 226 matrix representing the fitted curve defined by the 9 parameters.

The fitted image is then compared to the original scanned image, which is produced by plotting the 1 x 226 data points as discussed above, to measure the performance of the curve-fit function. This is accomplished by plotting the fitted image against the scanned image and solving the linear regression through these values. The fitted image is then compared to the original image by plotting the fitted image against the scanned image and solving the linear

regression through the values. The fit must meet predetermined validation criteria, which are determined empirically, as discussed below. An exact match between the scanned and fitted image would yield a line with slope = 1 and r-square (r^2) = 1.

Once the curves have been fitted, the peak height of the curve in the detection zone is determined by subtracting the parabolic baseline from the maximum peak height. The peak height is then compared to that of a previously run sample of known analyte concentration. If the peak height of the clinical sample is greater that the peak height of the reference sample, then the test result is positive. If not, a negative test result is returned. The peak height of the curve representing the control zone may also be checked to determine if it meets a required minimum height, in order to test that the system is functioning.

Alternatively, peak areas may be calculated and compared to give a determination of analyte concentration in the sample. The graph may be mathematically analyzed, with a sigmoidal calculation across the background and a gaussian calculation to integrate the area beneath each of the two peaks. The analyte concentration is then determined based upon the ratio of the integrated area beneath each of the two peaks.

Methods for Reducing the Image to Parameters

Images or large sets of data, are not readily amenable for developing and training neural net analyses. For large data sets, the number of inputs required for neural network training must be reduced. To do this assumptions regarding the types of data that can be omitted are made. As a result of the loss of information, the performance of subsequently trained neural nets will hinge on the validity of the assumptions made. A method for reduction of data that reduces dimensionality with minimal or no loss of information will avoid this problem. The reduced database can be validated by using it to reconstruct the original dataset. With minimal or no loss of information, subsequently trained networks should yield higher performance than networks that are trained with less complete data. Methods are provided herein for reducing dimensionality with minimal loss of information. These methods are directly applicable to the

images that are generated and data generated from the test strips described herein and is also generally applicable to all images and large datasets.

Methods for Optimizing the Reconstructed Image

Parameters for a mathematical function designed to reproduce, or approximate the scanned image are effective at determining the concentration of the compound being tested and thereby providing a means to classify the sample being tested. Examination of the data, for example, from the fFN test provided herein demonstrates that a scanned image can be constructed from three basic elements. There is a background density, referred to herein as the baseline density. Superimposed on the baseline are the two peaks. The first peak is referred to as the control peak and the second is the test peak. Since the shape of these peaks is very similar to a normal curve, it was assumed that the peaks have a gaussian shape. One characteristic of the "normal curve" that can be exploited is that the area under the curve is always 1.0. By modifying the formula, the height of a peak can be determined from a single function parameter.

When analyzing an image, the peak density function used is:

Peak Density = Height * EXP(-Z*Z)

where Z = (X - Pos) * S,

X = Pixel Number,

Pos = Pixel number of center of peak,

S = Spread or width of the peak, and

Height = Height of the peak.

This function contains three parameters, Height, S and Pos. When the three parameters are set correctly, this function will closely match one of the peaks in the test strip image. With two peaks in the image, this function can also be used to estimate the second peak. With two peaks, there are six parameters so far that must be optimized. The goal of the optimization will be to change the above parameters in such a way as to reconstruct the image as closely as possible.

In order to reconstruct the image completely, the baseline of the image must also be estimated. Examination of scanned images showed that the

baseline had a slight curve. By using a parabolic or quadratic form function, the baseline density is estimated. The function for the base density is,

Base Density = X * X * Curve + X * Slope + Offset.

Thus, the image can be accurately reconstructed by combining these three function in the following summation,

Image Density = Base Density + Control Peak Density + Test Peak Density.

This results in a total of nine parameters that must be optimized for an accurate reconstruction of the image.

The basic problem with attempting to fit this complex function to the test strip image is that there are no simple means for finding the optimal values for function parameters as there are for linear regression. There are many numerical techniques that can be used to optimize the parameters of the above image density function, for example, the downhill simplex method (see, "Numerical Recipes in C", Second Edition, Cambridge University Press, 1992).

The basic method of optimization uses an iterative approach to optimize the function parameters based on a defined cost function. Here the cost function is defined as the sum of the squares of the differences between the original image and the reconstructed image for every pixel in the scanned image. The downhill simplex method uses a simplex to accomplish this optimization. A simplex is a geometrical figure in N dimensions containing $N+1$ points. For the image density function defined above, N has the value 9. In two dimensions, for example, a simplex will contain 3 points, with lines connecting each pair of points. This simplex is called a triangle. As the dimension increases, the complexity of the simplex also increases. In three dimensions a simplex is a tetrahedron. This implies that if there are N parameters to be optimized, then $N+1$ solutions must be maintained. This translates to N^2+N storage locations that are required to run the algorithm.

For exemplification, the optimization problem with 2 parameters is as follows. The simplex, a triangle, is formed from three points or three different sets of values for the parameters. These three points (call them solutions A, B and C), which are generated in the following way. Starting with and initial set of parameters (solution A), each parameter is perturbed by a small amount

(typically 0.01). When the first of the two parameters is changed, solution B is generated. When the second parameter is perturbed, solution C is generated. The three solutions must be evaluated to determine the error function value for each.

Suppose that solution A has the highest error function value. The simplex algorithm attempts to make an improvement by picking a new point (solution, or set of parameters), that lowers the error function value. This basic operation is called a reflection. Three attempts are made at improving the solution. The first, normal, reflection picks its new set of parameters by forming a line from point A to the average of the remaining points. The line is then extended through the average point an equal distance. This new point is the reflection point. Reflection is the correct term since if one were to place a mirror on the line between B and C, the new point corresponds exactly to the reflection of A in the mirror.

If the new error function value for the normal reflection is better than the best current solution, then an expansion reflection is attempted. In this case the line from A is extended by the Step Up Factor (typically 1.50) through the average point. This operation makes the simplex larger. The point that gave the best error function value (either the normal reflection of A or the Expansion reflection of A) is retained as the new A point.

If the new error function value for the normal reflection is still the worst solution, then a contraction reflection is attempted. In this case the line from A is extended by the Step Down Factor (typically 0.75) through the average point. This operation makes the simplex smaller. If this solution is better then the original error function value for point A, the reflection point is retained as point A. If no improvement is made in the A solution, then the entire simples is contracted by moving each point toward the point with the best error function value by the fraction specified by the Shrink Factor (typically 0.95). These reflection operations continue until the difference between the best and worst solutions falls below the Restart Tolerance (typically 1.0E-9).

Alternative Method 1 for R Reducing the Image to Parameters Using a Neural Network

A neural network can be used as an alternative to a polynomial mathematical function for the purpose of generating parameters that can be used to reconstruct an image. The basic architecture of the neural network contains at least three processing layers. During the training process, a sequence of example images are presented to the network for training. Training continues so that the error between each image and its reconstruction is minimized across the set of images used for training. The image, or a subsection of the image, is presented to the input layer of the network. The middle layer, or hidden layer, of the network contains a number of processing element that is much smaller then the number of inputs in the input layer. The output layer contains the same number of processing elements as the input layer. The output layer of the network will represent the reconstructed image that is presented to the input layer.

An alternative architecture contains an odd number of hidden layers, with the middle hidden layer containing a much smaller number of processing elements than the input and output layers. In each layer of the network, each processing element is connected to each of the processing element outputs of the previous layer.

The processing element used in the network typically generates a weighted sum of the inputs to processing element, with a transfer function applied to the weighted sum to generate the output of the processing element. The transfer function is any such function normally used in a neural network, including the sigmoid function, or the hyperbolic tangent function.

The neural network can be trained using any standard neural network training rule, including the back propagation learning rule. At each step of the training process, a training image is presented to the inputs of the neural network. The same image is also be presented to the outputs of the network as the desired, or target, output of the network. As learning proceeds, the error between the outputs of the neural network and the desired outputs of the network decreases.

In order for the error to decrease, the neural network middle hidden layer generates a greatly reduced representation of the input image that contains

enough information to reconstruct the image. This reduced representation therefore also contains the information needed to classify the image.

Once trained, a new image is presented to the inputs of the neural network. The outputs of the middle hidden layer are then be used as the inputs to the classification means for further processing.

Alternative Method 2 for Reducing the Image to Parameters Using a Neural Network

A second alternative method for reducing an image to useful parameters is to substitute the neural network directly in place of the polynomial mathematical function. Here, the inputs of the neural network are the coordinates of the pixel in the image being examined. The desired output of the network are the density value of the associated pixel.

The architecture of this neural network is substantially smaller then the architecture described in the first alternative method. Here the weights of the neural network become the parameters to be used by the classifier. The types of processing elements used in this architecture include the radial basis function type, and provisions might be made to allow a mix of processing element types in the hidden layer of the neural network. The architecture is developed to provide the smallest possible number of weights while still being capable of reconstructing the image.

In this alternative, the neural network is trained only on the image under consideration. Thus, each time a sample is tested, the network would be retrained. The weights of the trained network are used as inputs to the classification means.

Assay Validation

Method for Classifying the Image from the Parameters

Once the parameters are estimated, the parameters generated from the image reconstruction process along with several parameters easily calculated from the scanned image are used to classify the sample. In addition, the image parameters from several reference scans are used. The process of classification incorporates two steps. The first is a validation step to determine if the sample

under consideration should be rejected or classified. The validated result is then classified as positive or negative as described above.

To ensure the accuracy of a test result, the system producing that result should be validated. Validation protocols are used to confirm that all components of a system operate properly, and that the data received from the system is meaningful. Moreover, in systems where raw data from instruments are manipulated by software, the proper functioning of that software should also be validated.

Validation of data analysis software can be performed in any number of ways. Typically, a known sample (e.g., reference, positive control, negative control) can be tested in the system to confirm that the expected result is obtained. Alternatively, known raw data can be stored in memory and acted upon by the data analysis software to confirm that the expected result is obtained. Such validation protocols ensure that the software is operating properly before a clinical sample of interest is evaluated in that system.

Validation of test systems can also be performed during the evaluation of a clinical sample being tested by that system. These types of validation protocols can evaluate components of the system, either individually or together. When the criteria set by assay validation protocols are not achieved, an invalid result is obtained, and the user will be made aware of the system malfunction. Such processes ensure that only accurate test results are presented to the user.

In an exemplary embodiment, for example, data are validated by several methods. First, the data are checked for completeness by checking that the size of the matrix is m x n, where m is the number of columns of data collected (e.g., location on dipstick, dark reading, reflectance at λ_1 and reflectance at λ_2) and n is the number of steps, or readings, taken along the test strip. For example, in the preferred embodiment, the matrix must be of an exact size of 4 x 226.

The extent of color development at the control line may also be used to validate that each assay ran properly. For example, quantitation of the control peak height, or area, may be used as assay validation criteria. Specifically, an

assay validation criterion might be imposed such that only those scans that yield a control peak height of greater than or equal to a specified value, determined empirically for the assay in question, will be valid, otherwise the scan is rejected. For example, in a particular embodiment, where fFN is detected on an immunoassay test strip, as described herein, the control peak height must be greater than 0.05 intensity units.

Method for generating controls for assays and the resulting controls

A method for generating controls and the resulting controls are provided herein. This method is based upon the observation that some systems generate data that are conducive can be modelling (i.e., fitting). Empirical analysis will deterimine construction of an algorithm such that valid data are modelled accurately, while invalid data are difficult to model. For example, if it is known that a scan by the reader (intensity versus distance along the strip) should be relatively flat except for two normally distributed peaks in the central region. , It has been found that the curve is composed of a linear, or parabolic, function to estimate the baseline, a first gaussian function to estimate one of the peaks, and a second gaussian function to estimate the other peak. Such a composite function would readily fit scans generated during normal operation, but would not fit well scans generated during abnormal operation. Under these types of conditions, the comparison of the fitted image with the original image may serve as another criteria for assay validation.

More specifically, if the linear regression of the fitted versus original image were solved, the r-square value and the slope of the linear regression may serve as assay validation criteria. Thus, based on historical empirical data obtained from other runs on similar samples, upper and lower limits on r-square and slope would be set such that scans which do not fit within the limits within a specified number of attempts to optimize the fit are rejected. For example, assay validation criteria might include a requirement that r-square be greater than or equal to 0.97. The particular value is dependent upon the test and will be determined by the skilled practitioner. Moreover, another assay validation criteria might include a requirement that the slope of the linear regression must be between 0.98 and 1.10, inclusive. Again the particulars of each determina-

tion are a function of the test, the type of data and results, and the tolerance for and amount of variation.

This method is advantageous in that it provides an internal validation, in contrast with other controls, which usually involve comparison of results against a previously established range, or against other test samples, such as positive or negative controls or reference samples. It is based on the concept that any assay can be controlled based on expected results.

For example, using the assay exemplified herein, where fFN is detected in the immunoassay, based upon repeated performance of the assay, it has been found that the expected reflectance data can be readily modeled by a composite curve comprised of a parabolic and two gaussian functions. Therefore, any time the assay is run, the resulting data should be modeled (fit) by the same composite curve. Knowing this, it is possible to identify scans, which although the positive and negative controls are show tha the assay worked, should be classified as invalid. Such scans (assay runs), for example, if they required a sigmoidal, rather than parabolic, baseline. would not readily fit (model) with the composite function, and would be classified as invalid. The failure to fit for example, could reflect defects in the test strip, or changes in the environment during the run, etc., that would affect the run, but might not affect the positive and negative controls.

If assay validation criteria have been met, then the result can be interpreted as either positive or negative by comparing the intensity of the test peak of the clinical sample with the intensity of the test peak of the positive reference. A test is positive if the intensity of the test peak of the clinical sample is greater than or equal to the intensity of the test peak of the positive reference. A test is negative if the intensity of the clinical sample is less than the intensity of the test peak of the positive reference. In calculating intensity, either peak height or peak area may be used.

Alternative Method for Classifying the Image Using a Neural Network

Based on the available data generated from scans all possible variables were identified that could be used to improve the ability to classify the sample.

The initial training runs used the parameters generated from the image reconstruction process along with several parameters easily calculated from the scanned image. One such parameter is the area under a peak. It can be calculated from original parameters as following:

Area = sqrt(π) * Height / S, where S is spread or width of the peak. A sigma variable, related to the normal distribution can also be calculated from the parameters by:

Sigma = 1 / (sqrt(2) * S).

In addition, the image parameters from a Calibrator scan (fFN positive reference) were also used. The following is a list of the variables that are available for use by the neural network.

1. Sample Baseline Square Term
2. Sample Baseline Linear Term
3. Sample Baseline Offset
4. Sample Control Peak Position
5. Sample Control Peak Sigma
6. Sample Control Peak Area
7. Sample Test Peak Position
8. Sample Test Peak Sigma
9. Sample Test Peak Area
10. Sample Test Peak Height
11. Sample Control Peak Height
12. Sample Baseline estimated value between the peaks
13. Sample Ratio of Test Area to Control Area
14. Sample Ratio of Test Height to Control Height
15. Calibrator Baseline Square Term
16. Calibrator Baseline Linear Term
17. Calibrator Baseline Offset
18. Calibrator Control Peak Position
19. Calibrator Control Peak Sigma
20. Calibrator Control Peak Area
21. Calibrator Test Peak Position

22. Calibrator Test Peak Sigma
23. Calibrator Test Peak Area
24. Calibrator Test Peak Height
25. Calibrator Control Peak Height
26. Calibrator Baseline estimated value between the peaks
27. Calibrator Ratio of Test Area to Control Area
28. Calibrator Ratio of Test Height to Control Height

Four predictor variables were also added. In these variables the calibrator strip value is compared to the sample strip value and a +1 or −1 is used depending on the comparison. The additional variables are:

Test Area Predictor

Area Ratio Predictor

Test Height Predictor

Height Ratio Predictor.

The desired, or target output of the neural network was a classification of the concentration of the sample. If the sample concentration was greater than or equal to 50 ng/ml the desired output was set to 1.0. The desired output was set to 0 otherwise. A sensitivity analysis of the associated training runs was used to indicate which variables were important to the prediction task. The ThinksPro software product from Logical Designs Consulting was used to train the networks and perform the sensitivity analysis. Alternatively, a variable selection process based on genetic algorithms or some other method could be used to select the best subset of variables from this list (see, e.g., copending U.S. application Serial Nos. 08/798,306 and 08/912,133, which describe a suitable variable selection process).

Using the reduced set of variables one or more networks are trained to estimate the classification of the sample. If more than one network is used, the outputs of each network are averaged together to give a consensus result.

In another embodiment, the nine variables may optionally be fed through a previously trained neural network to obtain a test result. For example, the nets may be trained with data for which ELISA test results are known. Alternatively, variables other than the nine described above may be used to

train the neural net. The nets can be used not only to return positive or negative results, but also to determine if the assay itself is valid for any particular run.

The reduction of data for input to neural networks can be accomplished by a neural network itself. An example of such a net is a net with an hourglass architecture with an input, output and three hidden layers, wherein the input and output layers contain n nodes, with the first and third hidden layers containing less than n nodes, and the second hidden layer containing five nodes. If trained so that the output layer exactly matches the input layer, such nets would reduce the original dataset of n elements down to five elements, and also retain the ability to reconstruct the original dataset of n elements from these five elements.

Further Analysis Using Decision Support Systems

The output from the data analysis step provides an assessment of the raw biochemical test data that is measured by the reader or other instrument. Such data may then be considered as is, but is can be further entered into a decision-support system, particularly a neural network, that has been trained to evaluate the particular data and disease. For example, U.S. application Serial No. 08/599,275, now abandoned, copending U.S. application Serial No. 08/798,306, and copending U.S. application Serial No. 08/912,133, filed 8/14/97, as well as published International PCT application No. WO 97/29447 and International PCT application No. PCT/US98/16891, which claims priority to U.S. application Serial No. 09/134,636 and U.S. application Serial No. 08/912,133. PCT application No. WO 97/29447 claims priority to U.S. application Serial No. 08/599,275, filed 2/9/96, now abandoned, and copending U.S. application Serial No. 08/798,306. These documents provide neural nets and methods for developing neural networks for diagnosis of disorders that can be used in the systems as provided herein.

The accuracy of biochemical test data is improved when used in these neural nets. Such neural nets, are thus contemplated for inclusion in the systems herein.

Briefly, in the methods described in these applications patient data or information, typically patient history or clinical data, are analyzed by the decision-support systems to identify important or relevant variables and decision-support systems are trained on the patient data. Patient data are augmented by biochemical test data or results to refine performance. The resulting decision-support systems are employed to evaluate specific observation values and test data to guide the development of biochemical or other diagnostic tests, to assess a course of treatment, to identify new diagnostic tests and disease markers, to identify useful therapies, and to provide the decision-support functionality for the test. Methods for identification of important input variables for a medical diagnostic tests for use in training the decision-support systems to guide the development of the tests, for improving the sensitivity and specificity of such tests, and for selecting diagnostic tests that improve overall diagnosis of, or potential for, a disease state and that permit the effectiveness of a selected therapeutic protocol to be assessed are also provided. The methods for identification can be applied in any field in which statistics are used to determine outcomes. A method for evaluating the effectiveness of any given diagnostic test is also provided.

Thus, such neural networks or other decision-support systems will be included in the systems provided herein as a means of improving performance of the biochemical test data.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

Immunoassay Test Strip

A. The Test Strip

The test strip **100** includes a membrane system including three components: a porous or bibulous member **102**; a conjugate pad **108**; and an absorbent pad **110**. The membrane system may be mounted on a substrate or backing **112**, with the conjugate pad **108** and the absorbent pad **110** slightly overlapping the porous or bibulous member **102**, which is interposed thereinbetween. As can be seen, the conjugate pad **108** overlaps the porous or

bibulous member **102** so that a fluid sample placed onto the conjugate pad **108** is communicated from the conjugate pad **108** to the porous or bibulous member **102**. Similarly, the absorbent pad **110** overlaps with the porous or bibulous member **102** so that fluid samples introduced into the porous or bibulous member **102** from the conjugate pad **108** can then be transmitted to the absorbent pad **110**. Thus, the conjugate pad **108**, the absorbent pad **110** and the porous or bibulous member **102** are all in fluid communication with one another, making any fluid sample placed on the conjugate pad **108** able to propagate through the conjugate pad **108** to the porous or bibulous member **110** and then to the absorbent pad **110**.

The porous or bibulous member is capable of transporting a liquid sample along the test strip and serves as the solid support upon which the immunoreactions occur. Antibodies which react with the target analyte and/or label are immobilized on the solid support. Possible solid supports include paper and cellulose derivatives, such as cellulose esters and ethers, natural and synthetic polymeric materials, such as vinyl polymers and partially hydrolyzed derivatives, polycondensates, copolymers and inorganic materials. A preferred solid support is a nitrocellulose membrane.

The porous or bibulous member contains two distinct zones, a detection zone **104** and a control zone **106**, at which two different antibodies are immobilized. The detection zone contains an immobilized capture antibody that binds the analyte of interest, whereas the control zone contains an immobilized antibody or other component, such as an antigen, that binds labeled antibody conjugate (discussed below) which has not bound to analyte.

The membrane system also includes a conjugate pad **108**, which serves as a sample application component, and which includes an antibody to the analyte, which is conjugated to a detectable label. The conjugate pad is in fluid communication with the porous or bibulous member **102**. The labeled antibody conjugate is diffusively bound to the conjugate pad and becomes mobile upon application of the liquid sample and moves along the test strip. The conjugate pad is made of a porous material, such as glass fiber. The conjugate pad may also act as a pre-filter for the sample.

The membrane system may also include an absorbent pad **112**, which is also in fluid communication with the porous or bibulous member, and which serves to draw liquid continuously through the device. The absorbent strip may be made of a material such as cellulose paper or other material known to those of skill in the art.

Referring to FIG. 2A, which depicts an exemplary immunoassay device, including a test strip and housing assembly **200**, the housing **202** generally surrounds the test strip **100** (FIG. 1) and includes an opening through which test sample is applied **204**, as well as an aperture above the detection and control zones **206** that permits measurement of the amount of label by the reader, which is correlated with the amount of analyte in the test sample. The housing **202** includes at its upper surface **208** a fattened end **210**, used for gripping the housing **202**, an application window **204** (or sample window) through which a sample is applied to a conjugate pad **108** of an immunoassay test strip within the housing **202**. The housing **202** also includes a test window **214** through which the test result of the immunoassay is viewed. In accordance with the embodiments shown, no window material is mounted within the test window **214** (or the sample window **212**). Thus, an optical path from outside the housing **202** through the test window **214** to the immunoassay test strip is unobscured by even a transparent material. Other alternative embodiments may include an optically transparent material (transparent at wavelengths emitted by light emitted from devices described herein), however, such is not preferred. Also, as shown in FIG. 2A and FIG. 2B, the housing may include a symbology, exemplified as a bar code **216** or **316** that can be read by the reader or a separate reading device and associated with identifying information pertaining to the particular device and/or test run or other information.

An alternative embodiment of the test device is shown in FIG. 2B. The components of device are shown in FIG. 3 and include the upper and lower members **302** and **304** of the housing and the test strip **100**. Also shown are the sample application port **306**, test window **308**, and the optionally included bar code **316**.

Referring next to FIG. 4 a top view is shown of the immunoassay test strip housing **202** of FIG. 2A. Shown are the sample window **212**, and the test window **214**, and the enlarged gripping portion **210**. Also shown are structures **402** for holding the immunoassay test assembly within the housing **202** and structures **404** for securing upper and lower halves of the housing **202** to one another.

Referring next to FIG. 5, a side cross-sectional assembly view is shown of the housing **202** for the immunoassay test strip **100**. Shown are the sample window **212**, the test window **214**, and the structures **402** for holding the immunoassay test strip assembly in place within the housing **202**. As can be seen, an upper half **502** of the housing **202** is mated with a lower half **504** of the housing **202**. The immunoassay test strip is sandwiched between the upper and lower halves **502** and **504** of the housing **202** and is secured in place by the structures **402** of the upper half **502**. The immunoassay test strip is positioned so as to be viewable through the test window **214** when the immunoassay test strip assembly is secured within the housing and the conjugate pad is positioned to be contactable through the sample window **212**.

These devices are particularly adapted for use with the reflectance reader provided herein.

B. Colored Latex Label and assays using the latex label

The immunoassay test strip includes a membrane system supported on a plastic backing. The membrane system is formed from three partially overlapping materials: a conjugate pad made of Whatman glass fiber (F075-07S, 2.4 cm length) treated with polyvinyl alcohol (PVA), a nitrocellulose strip supplied by Sartorius (8 μm, 3.3 cm length) and an absorbent pad made of Whatman C7218 (1.65 cm length) cellulose paper. These three materials are in fluid communication with each other. The conjugate pad and nitrocellulose overlap by 1 mm and the nitrocellulose and absorbent pad overlap by 4 mm. The membrane materials are hand-laminated and attached to a membrane card, which is cut using an Azco guillotine compression cutter, using G&L adhesive membrane.

The conjugate pad contains a mouse monoclonal anti-fFN antibody (FDC-6 or A137) conjugated to latex particles containing a blue dye. The conjugate pad acts as a pre-filter for the sample in that mucous from the sample is left behind in the conjugate pad after performing the assay.

The latex particles, which are polymerized from styrene and acrylic acid, may be any suitable latex particles (such as are available from Bangs Laboratories). The particles are polymerized in an aqueous solution with a surfactant added. The particles are internally labeled with blue dye by swelling the particles in organic solvent and adding the dye. The particles are then placed in an aqueous solvent, which shrinks the particles and traps the dye. The dye is an organic soluble blue dye. Carboxyl groups are covalently attached to the surface of the bead for coupling to the antibody. The particles (Bangs Uniform Microsphere Stock D0004031CB) are supplied as a 2.5-10% aqueous suspension containing surfactant and have a mean diameter of 0.40 μm, with a standard deviation of 0.4 μm, and a surface area of 1.405e+13 $\mu m^2/g$.

Antibodies are conjugated to the latex particles in a one-step covalent conjugation process using EDAC, a carbodiimide coupling reagent. The conjugate is characterized as approximately 1% solids; 50 μg/mg beads total bound protein (Bead BCA); and greater than 50%, preferably 80%, covalent bound protein (Tris-SDS + Bead BCA).

The antibody conjugated to the latex particles is mouse monoclonal antibody specific for fetal fibronectin. The antibody (FDC-6 or A137 monoclonal, deposited at the American Type Culture Collection as accession number ATCC HB 9018(see U.S. Patent No. 4,894,326) is raised against whole molecule onco-fetal fibronectin from a tumor cell line. The antibody is produced as ascites and is purified by Protein G and dialyzed into PBS buffer.

The nitrocellulose strip contains two distinct zones, a detection zone and a control zone, at which two different antibodies are immobilized. The detection zone contains immobilized anti-fibronectin polyclonal antibody as a capture antibody, whereas the control zone contains immobilized goat anti-mouse polyclonal antibody. The anti-fibronectin polyclonal antibody is produced

in goats. The antisera is obtained from a commercial source and is purified by use of a fibronectin column which is made by attaching purified fibronectin (antigen) to a resin. The antisera is passed through a column containing the resin. After washing unbound material, the antibody is eluted via low pH glycine. The purified antibody is then dialyzed. The purified goat anti-mouse IgG antibody (GAMGG) immobilized in the control zone is obtained from a commercial source (e.g. Biosource) or can be prepared, if desired, e.g. by passing the serum through a mouse IgG column, which binds the antibody, and then eluting off the antibody using glycine, or by any suitable method.

The antibodies are applied to the conjugate pad and nitrocellulose strip using an IVEK Linear Striper, which is a volumetric ceramic piston pump dispenser. The anti-fibronectin polyclonal capture antibody is applied in a 1x Spotting Buffer P/N 00387, which contains citrate, phosphate and NaCl, at an antibody concentration of 1 mg/ml and a striping rate of 1 μl/sec. The position of the test line is about 37-40 mm, preferably 38-39 mm, from the bottom of the strip. The control antibody is applied in a 1x Spotting Buffer P/N 00387 at a concentration of 0.5 mg/ml and a striping rate of 1 μl/sec. The position of the control line is approximately 43-46 mm, preferably 44.5-45.5 mm, from the bottom of the strip. The dimensions of the antibody stripes are approximately 8.1 mm (wide) x 0.5-1.0 mm (high), or any other suitable size. The nitrocellulose strip is not otherwise treated after application of the capture and control antibodies to block non-specific binding sites.

The detection and control stripes are applied to the strip and then dried for at least 24 hours at RT, after which the conjugate is striped onto the strip. The conjugate is mixed in a diluent containing 20% sucrose, 0.2% BSA, 0.05% TW20 and 0.05% sodium azide in 100 mM Tris. After application of the conjugate, the strip is then dried, preferably for 30 minutes at 37°C, or at least 24 hours at RT, or the equivalent thereof.

The test strip is contained within a housing, which includes a lower member and an upper member with openings that include a circular aperture above the area of the conjugate pad, through which test sample is applied, and a rectilinear aperture above the detection and control zones. The circular

application aperture is in contact with the test strip. The latex conjugate is placed slightly downstream from the sample application opening. The upper and lower members are mated together to sandwich the test strip. The test strip is confined non-removably in the housing, and the device is not intended to be re-usable. The upper member is configured for use with a reader that measures the amount of label that is indicative of the amount of fetal fibronectin in the test sample.

C. Colloidal Gold Label and assays using colloidal gold label

In an alternative embodiment, colloidal gold is used for labeling the antibody. The test strip configuration is similar to that described in EXAMPLE 1A for the latex particle embodiment, with the following modifications.

In the colloidal gold assay, a goat polyclonal antibody to human adult and fetal fibronectin is present in the conjugate pad, immobilized mouse monoclonal anti-fetal fibronectin antibody (specific to the III CS region of fetal fibronectin) is present in the detection zone of the nitrocellulose test strip and immobilized human adult fibronectin is present in the control zone.

The anti-fibronectin antibodies (polyclonal) are labeled with colloidal gold by passively adsorbing anti-fibronectin antibodies onto colloidal gold. This preparation is then treated with a solution containing protein and polyvinyl pyrrolidone (PVP) to coat the colloidal gold particles. This method is described in Geoghegan and Ackerman, Journal of Histochemistry and Cytochemistry, 25(11):1187-1200 (1977).

EXAMPLE 2

Immunoassay Procedure

A. Colored Latex Label

In conducting the assay, the sample is extracted from a swab into antiprotease transfer buffer (0.05 M Tris buffer, pH 7.4, 1% BSA, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 500 Kallikrein Units /ml of Aprotinin), heated for 15 minutes at 37°C and filtered through a large pore (25 μ) plunger filter. A volume of 200 μl of test sample is then delivered to the conjugate pad at the application zone using a standard plastic pipet. Any fFN in the sample will bind to the labeled monoclonal antibody and the resulting

complex migrates into the nitrocellulose strip. When the complex encounters the detection zone, the immobilized anti-FN antibody binds the complex, thereby forming a colored stripe due to the accumulation of the dye-containing latex beads. Any unbound latex-conjugated anti-fFN antibody continues to migrate into the control zone where it is captured by the immobilized goat anti-mouse antibody and thereby forms a colored stripe due to the accumulation of the dye-containing latex beads. The reaction time is 20 minutes.

B. Colloidal Gold Label

The test strip assay procedure is similar to that described in EXAMPLE 2A for the latex particle embodiment, with the following modifications. The buffer used to extract the sample is Tris-acetate and a protein matrix (4% PSA and 4% PVP).

Fetal fibronectin and adult human fibronectin in the sample bind with the labeled anti-fibronectin antibody conjugate on the conjugate pad. The labeled fetal fibronectin-anti-fibronectin complex and adult human fibronectin-anti-fibronectin complexes, and unbound labeled anti-fibronectin conjugate migrate into the nitrocellulose strip, where they encounter the detection region, including immobilized anti-fetal fibronectin monoclonal antibody.

In the detection region, the immobilized anti-fetal fibronectin capture antibody binds with the fetal fibronectin-anti-fibronectin complex, whereby a gold label forms a colored stripe due to concentration of the gold label. The amount of gold label bound to the test region correlates with the amount of fetal fibronectin in the sample.

The unbound labeled anti-fibronectin antibody conjugate and adult human fibronectin-anti-fibronectin complex then pass to the control region of the immunoassay test strip, which includes immobilized adult human fibronectin. There, any unbound antibody conjugate binds to the immobilized adult human fibronectin, where the gold label forms a second colored stripe. The presence of a colored stripe indicates that the assay results are valid, whereas the absence of this stripe indicates that the results are not valid, i.e., that the sample did not reach the control region, and thus a good reading at the test

region cannot be assumed. Any adult human fibronectin-anti-fibronectin complexes formed do not bind with the detection or control zones.

EXAMPLE 3

Operation of the Reflectance Reader

The test strip is read using the reflectance reader exemplified herein. This reader (described above) is adapted to read an immunochromatographic test strip supported within the housing. The reflectance reader includes a cassette slot for receiving the test-strip housing, and a sensing head assembly for reading the test strip supported within the test-strip housing using reflected light. The sensing head assembly includes a first light-emitting diode (LED_1), a second LED (LED_2), a silicon photodiode detector, and 39 optical fibers randomly arranged in a narrow slit (e.g., 0.020" wide) located at the bottom of the sensing head assembly. LED_1 emits light with a wavelength of 430 nm (blue), and LED_2 emits light with a wavelength of 595 nm (amber). The optical fibers are arranged in three groups of 13 optical fibers each: the first group transmits light emitted by LED_1 to the slit; the second group transmits light emitted by LED_2 to the slit; and the third group transmits reflected light received at the slit to the photodetector. The 39 fibers each include an end randomly arranged within a plane located at the slit such that the ends are co-planar, with the plane being normal to the test strip when the sensing head assembly is positioned (as described below) to take reflectance readings. The optical fibers in each of the three groups are randomly arranged within the plane with respect to the fibers of the other two groups.

The slit width is selected to be as narrow as permitted, with the practical minimum being driven by the availability of small diameter optical fibers. The maximum slit width should not be larger than about 90% of the width of the colored stripe, otherwise the background of the strip, in addition to the colored stripe, will be read and less color will be detected, unless the slit, or aperture, is positioned directly above the colored stripe.

When the housing is inserted into the cassette slot of the reader, a spring mechanism rotates the sensing head directly over the second aperture of the housing such that the plane defined by the optical fibers is normal to the

surface of the nitrocellulose strip at a distance of about 0.010". Light from LED_1 and LED_2 can be transmitted by the fibers onto the nitrocellulose strip at a normal angle, and light reflected normally from the strip can be transmitted by the fibers to the photodetector.

The sensing head takes three separate reflectance readings of each portion of the nitrocellulose strip by reading the output of the photosensor while controlling LED_1 and LED_2. The first reading, used to determine the amount of ambient light leaking into the reader (e.g., light leaking through the slot entrance, or light reflected into the reader through the white plastic of the housing), is a dark reading taken with LED_1 and LED_2 both turned off. The dark reading count is subtracted from the other two readings to correct for light leakage. The second reading, used to determine background reflections associated with the nitrocellulose, is taken with LED_1 pulsed on and LED_2 turned off. The third reading, used to detect the presence of the latex label on the test strip, is taken with LED_2 pulsed on and LED_1 turned off. A control circuit reads the photodetector output and controls the on and off operation of LED_1 and LED_2. A memory circuit stores the raw and/or processed data. The data may also be displayed to the operator via an appropriate interface (e.g., an alphanumeric character matrix).

After being positioned above the housing by the spring mechanism, the sensing head can be moved slidably across the test strip to allow the head to scan the entire exposed surface of the nitrocellulose strip (including the detection and control zones). In the preferred embodiment, this distance is approximately 0.452". The head is slidably connected to a rail (e.g., guide rods), and is coupled to a worm or screw gear driven by a stepper motor. Under the control of the control circuit, the stepper motor drives the head along the rail in small steps (e.g., 0.002"/step). At each step, the control circuit takes three readings as described above. Thus, the control circuit moves the head such that the optical fibers pass directly above and normal to the exposed surface of the nitrocellulose strip in a sequence of small steps, and takes a sequence of dark, LED_1 and LED_2 readings at each step. The control circuit then

processes the data read from the photodetector at each sequence of three readings to determine the presence or concentration of fFN.

Since the detection and control latex stripes are each about 0.020" wide, and since each step of the sensing head is about 0.002" long, there will be about 10 steps within each stripe. Thus, there will be 10 sets of three readings (i.e., dark, LED_1 and LED_2) at each of the stripes, and the remainder of the reading sets will not be made over either stripe.

The control circuit processes the LED_1 and LED_2 readings by first subtracting the "dark reading" taken at the corresponding step to correct for light leakage. The corrected LED_1 and LED_2 readings are then input into a ratiometric algorithm to determine the concentration of fFN. The algorithm is based upon the ratio of readings at the detection and control zones. If a sample includes a high concentration of fFN, latex readings at the detection zone will be relatively high and the readings at the control zone will be low. If the sample includes a low concentration of fFN, however, latex readings at the detection zone will be relatively low and readings at the control zone will be high. The algorithm calculates a reflectance ratio for each step which equals (amber count - dark count)/ (blue count - dark count). Generally, light leakage is so minimal that this step can be omitted. If the reflectance ratio is graphed as a function of the steps, the result will be a two-peak graph with the peaks occurring at the two stripes. The graph is mathematically analyzed, with a sigmoidal calculation across the background and a gaussian calculation to integrate the area beneath each of the two peaks. The fFN concentration is then determined based upon the ratio of the integrated area beneath each of the two peaks.

In operation, when the test-strip housing is inserted into the cassette slot of the reader, the sensing head rotates down over the exposed nitrocellulose strip, and the control circuit then moves the head to an initial position. The control circuit moves the head across the exposed surface of the nitrocellulose strip, including the detection and control zones, in small increments of 0.002" each. At each step, the control circuit takes a first reading of the photodetector output with LED_1 and LED_2 both off, takes a second reading with LED_1 pulsed on and LED_2 off, and takes a third reading with LED_1 off and LED_2 pulsed on.

The control circuit then steps the sensing head and repeats the three readings. This process is repeated for each of 226 steps (0.452" at 0.002"/step) until the entire surface is read. The control circuit may then analyze the raw data to determine the presence or concentration of fFN. The output values can be displayed via an operator interface, or can be output to another computer or apparatus.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

CLAIMS:

1. A point of care system for determining results from a diagnostic or risk assessment test, comprising:

a point of care test;

a reader for analyzing data from the test; and

software programmed for analyzing the results from the test.

2. The point of care system of claim 1, wherein the reader is a reflectance reader with a reader head that comprises:

a reader head body;

a light emitting diode;

a first fiberoptic bundle optically coupled to the light emitting diode;

a photodetector;

a second fiberoptic bundle optically coupled to the light photodetector;

an aperture in the reader head body;

a plurality of fiberoptic conductor ends arranged in a sigmoidal distribution in the aperture, a first portion of the fiberoptic conductor ends being of fiberoptic conductors of the first fiberoptic bundle, and a second portion of the fiberoptic conductor ends being of fiberoptic conductors of the second fiberoptic bundle.

3. The system of claim 1, wherein the point of care test includes a test strip that displays results from the test, and the reader is adapted to read the strip.

4. The point of care system of claim 1, wherein the reader is a reflectance reader, comprising:

a reader head comprising:

a reader head body;

a light emitting diode;

a first fiberoptic bundle optically coupled to the light emitting diode, and adapted to transmitting light form the light emitting diode;

a photodetector adapted for generating a reflection signal in response to reflected light;

a second fiberoptic bundle optically coupled to the light photodetector, and adapted to transmit an amount of reflected light to the photodetector;

an aperture in the reader head body; and

a plurality of fiberoptic conductor ends arranged in a sigmoidal distribution in the aperture, a first portion of the fiberoptic conductor ends being of fiberoptic conductors of the first fiberoptic bundle, and a second portion of the fiberoptic conductor ends being of fiberoptic conductors of the second fiberoptic bundle, the plurality of fiberoptic conductor ends being further arranged in a substantially co-planar relationship; and

a reader housing comprising:

a housing body; and

a cassette slot adapted to receive a test device.

5. The system of claim 1, wherein the reader comprises:

a control unit including a processor modified with a software subsystem, wherein the software is for analyzing the data produced in the test.

6. The system of claim 4, wherein the reader further comprises:

a control unit including a processor modified with a software subsystem, wherein the software is for analyzing the data produced in the test.

7. The system of claim 1, wherein in the point of care test is an immunoassay.

8. The system of claim 1, wherein the test assesses the risk of having a condition or diagnoses the condition.

9. The system of claim 1, wherein the condition is pregnancy-related or the test assesses fertility status.

10. The system of claim 1, wherein the condition is infertility-related, a neurological disorder, a cardiovascular disorder, an inflammatory disorder, a viral or bacterial infection, a hormonal disorder, a metabolic disorder or a genetic disease.

11. The system of claim 1, wherein the results of the test are qualitative.

12. The system of claim 1, wherein the results of the test are quantitative.

13. The system of claim 1, wherein:

the test is an immunoassay test strip, wherein the results from the immunoassay are detectable by a change in color or other property that can be detected using a reflectance reader; and the reader is a reflectance reader.

14. The system of claim 13, wherein the immunoassay test strip comprises:

(a) a conjugate pad which serves as a sample application component;

(b) a porous or bibulous member which is capable or transporting a liquid sample along the test strip and serves as the solid support upon which the immunoreactions occur; and

(c) an absorbent pad, which serves to draw liquid continuously through the device, wherein:

the materials of the membrane system form a single fluid flow pathway and the test strip is designed to be read by the reader.

15. The system of claim 14, wherein the porous or bibulous member comprises an immobilized capture antibody that binds to the analyte of interest in a detection zone.

16. The system of claim 8,

wherein:

the conjugate pad comprises an antibody diffusively bound thereto;

the antibody is bound to a target analyte and is labeled with a colorimetrically or fluorometrically detectable label.

17. The system of claim 1, wherein the reader is a reflectance reader.

18. The system of claim 17, wherein the reflectance reader comprises:

data processing software employing data reduction and curve fitting algorithms converting the reflectance signal obtained from reading the test strip into a determination of the presence of an analyte in a sample.

19. A method for determining results from an immunoassay, comprising:

(a) testing a patient sample for the presence of the a target analyte indicative of a condition or risk of having the condition by reacting the sample with an antibody specific for the analyte in a sandwich assay in which one of the antibodies is labeled with a colorimetrically detectable label;

(b) detecting the signal produced by the assay in a reflectance reader, wherein the signal is indicative of the presence of the analyte; and

(c) processing the data obtained from the reflectance signals using data processing software employing data reduction and curve fitting algorithms and/or a trained neural network to convert the reflectance signal obtained from reading the test strip into a data indicative of the presence or absence or a threshold concentration of analyte in a sample.

20. The method of claim 19, wherein the results are qualitative.

21. The method of claim 19, wherein the results are quantitative

22. The method of claim 19, wherein the data is input into a neural network trained for assessing risk related to or diagnosing the condition.

23. The method of claim 22, wherein the analyte is fetal fibronectin (fFN).

24. The method of claim 23, wherein the condition is pregnancy-related or the immunoassay assesses fertility status.

25. The method of claim 23, wherein the condition is an infertility-related disorder.

26. The method of claim 23 that assesses the risk of ectopic pregnancy, pre-eclampsia, infertility, preterm labor, imminent delivery, term induction, fetal membrane rupture or infections of concern during pregnancy that may comprise the pregnancy or harm the newborn, including, for example, ascending genital infections such as chlamydia or herpes simplex virus.

27. The system of claim 7, wherein the immunoassay detects fetal fibronectin (fFN) in a sample.

28. A reflectance reader, comprising:

a reader head comprising:

a reader head body;

a light emitting diode;

a first fiberoptic bundle optically coupled to the light emitting diode;

a photodetector;

a second fiberoptic bundle optically coupled to the light photodetector;

an aperture in the reader head body;

a plurality of fiberoptic conductor ends arranged in a sigmoidal distribution in the aperture, a first portion of the fiberoptic conductor ends being of fiberoptic conductors of the first fiberoptic bundle, and a second portion of the fiberoptic conductor ends being of fiberoptic conductors of the second fiberoptic bundle.

29. The reader of claim 28, that is adapted for reading an assay test strip.

30. The reader of claim 28, further comprising software for correlating the reading with a diagnostic parameter.

31. The reader of claim 30, wherein the software comprises a decision-support system.

32. The reader of claim 30, wherein the software comprises a neural network.

33. The reader of claim 32 including the plurality of fiberoptic conductor ends arranged in a substantially co-planar relationship.

34. The reader of claim 28 further comprising:

another light emitting diode;

a third fiberoptic bundle optically coupled to the other light emitting diode;

a third portion of the fiberoptic conductor ends being of fiberoptic conductors of the third fiberoptic bundle.

35. The reader of claim 34 including a bonding agent between and around the fiberoptic conductors at the fiberoptic conductor ends, wherein the bonding agent maintains the fiberoptic conductor ends in said sigmoidal distribution.

36. The reader of Claim 35, wherein said light emitting diode is adapted to emit light of a first wavelength, and wherein said other light emitting diode is adapted to emit light of a second wavelength.

37. A method for reading the surface of a test strip comprising an image, comprising:

scanning the reader head in the reader of claim 1 to a first position over the surface comprising the image;

determining a first amount of light reflected from the surface comprising the image;

illuminating the surface for light of a first wavelength, and determining a second amount of light reflected from the surface;

illuminating the surface for light of a second wavelength, and determining a third amount of light reflected from the surface; and

determining a parameter correlated with the intensity or shape of the image.

38. The method of claim 37, wherein the parameter is the amount of an analyte in a sample, which is a function of the first amount of light reflected, the second amount of light reflected, and the third amount of light reflected.

39. The method of claim 37, wherein the method is for determining an amount of an analyte in a sample by correlating the reading with the amount of analyte in the sample.

40. The method of claim 37, wherein the surface comprises an assay test strip.

41. The method of claim 37, wherein illuminating with light of said first wavelength includes illuminating with light of said first wavelength, wherein said first wavelength is selected to reflect substantially equally from all

regions of the immunoassay test strip, whereby said second amount of light is indicative of the immunoassay test strip.

42. The method of claim 37, wherein illuminating with light of said second wavelength includes illuminating with light of said second wavelength, wherein said second wavelength is selected to reflect substantially optimally from the test region of the immunoassay test strip, whereby said third amount of light is indicative of an amount of label at the test region of the immunoassay test strip.

43. The method of claim 38, wherein the analyte is fetal fibronectin.

44. A reader head for use in determining an amount of analyte in a sample comprising:

a reader head body;

a light emitting diode;

a first fiberoptic bundle optically coupled to the light emitting diode;

a photodetector;

a second fiberoptic bundle optically coupled to the light photodetector;

an aperture in the reader head body;

a plurality of fiberoptic conductor ends arranged in a sigmoidal distribution in the aperture, a first portion of the fiberoptic conductor ends being of fiberoptic conductors of the first fiberoptic bundle, and a second portion of the fiberoptic conductor ends being of fiberoptic conductors of the second fiberoptic bundle, the plurality of fiberoptic conductor ends being further arranged in a substantially co-planar relationship.

45. A reflectance reader, comprising:

the reader head of claim 44, wherein:

the first fiberoptic bundle optically coupled to the light emitting diode is adapted to transmitting light form the light emitting diode;

the photodetector is adapted for generating a reflection signal in response to reflected light;

the second fiberoptic bundle optically coupled to the light photodetector is adapted to transmit an amount of reflected light to the photodetector; and

the plurality of fiberoptic conductor ends are arranged in a sigmoidal distribution in the aperture, a first portion of the fiberoptic conductor ends being of fiberoptic conductors of the first fiberoptic bundle, and a second portion of the fiberoptic conductor ends being of fiberoptic conductors of the second fiberoptic bundle, the plurality of fiberoptic conductor ends being further arranged in a substantially co-planar relationship

46. The reader of claim 45, further comprising:

a reader housing comprising:

a housing body; and

a cassette slot adapted to receive a test device for measuring the analyte.

47. The reader of claim 45, that is for determining an amount of analyte in a sample

48. The reader of claim 45, wherein the test device is an immunoassay device.

49. The reader of claim 45, further comprising:

a control unit including a processor modified with a software subsystem.

50. The reader of claim 46, further comprising:

a control unit including a processor modified with a software subsystem.

51. The reader of claim 49, wherein said processor includes means for detecting the reflection signal and for generating an output signal indicative of an amount of analyte in response thereto.

52. The reader of claim 50, wherein the software comprises a decision-support system.

53. The reader of claim 52, wherein the decision-support system is a neural net.

54. A method for reading determining a risk or identifying a condition associated with the presence of fetal fibronectin in a cervicovaginal sample, comprising:

obtaining a cervicovaginal sample and reacting it with an antibody on a lateral flow test device to produce a stripe on the test device indicative of the presence of fFN in the sample;

reading the result with a reflectance reader that comprises a reader head by scanning the reader head to a first position over the test device;

determining a first amount of light reflected from the surface comprising the image;

illuminating the surface for light of a first wavelength, and determining a second amount of light reflected from the surface;

illuminating the surface for light of a second wavelength, and determining a third amount of light reflected from the surface; and

determining the amount of FFN in the sample.

55. The method of claim 54, further comprising, correlating the amount with the risk of developing a pregnancy related condition, with the presence of the condition, or with fertility status.

56. The method of claim 55, wherein the condition is selected from the group consisting of ectopic pregnancy, infertility, preterm labor, pre-eclampsia, imminent delivery, term induction and fetal membrane rupture and infections of concern during pregnancy that may comprise the pregnancy or harm the newborn, including, for example, ascending genital infections such as chlamydia or herpes simplex virus.

57. A method of classifying an image, comprising:

reducing the image to a set of derived parameters that are can be used to reconstruct the image within a specified degree of tolerance;

inputting the derived parameters into a classification means; and

determining the classification of the image based on the output of the classification means.

58. The method of claim 57, wherein the classification means is a neural network.

59. The method of claim 57, wherein the means of reducing the image to a set of derived parameters comprises:

defining a mathematical function containing a plurality of parameters representative of the image; and

optimizing the parameters of the mathematical function using a numerical technique that minimizes the error between the image and a reconstruction of the image using the mathematical function.

60. The method of claim 57, wherein the means of reducing the image to a set of derived parameters comprises:

inputting the image to a trained neural network, wherein the inputs to the network represent the image, the network comprises a hidden layer where the number of hidden elements is smaller than the number of inputs to the network, and the outputs of the network represent the reconstruction of the image; and

setting the derived parameters to the output values of the trained neural network.

61. The method of claim 57, wherein the means of reducing the image to a set of derived parameters comprises:

defining a neural network wherein the inputs to the network are the coordinates of a point in the image, a hidden layer contains a plurality of elements, and the output of the network represents the reconstruction of the associated point in the image;

training the neural network so that the error between the network output and the image are minimized for all points in the image; and

setting the derived parameters to the weights of the hidden layer of the trained neural network.

62. A method for determining results from a test strip, comprising:

(a) scanning the test strip in a reflectance reader to obtain a scanned image, wherein the signal is indicative of the presence of the analyte; and

(b) processing the data obtained from the reflectance signals using data processing software employing data reduction and curve fitting algorithms and/or a trained neural network to convert the reflectance signal obtained from reading the test strip into a data indicative of the test results presence or absence or a threshold concentration of analyte in a sample by a process that comprises:

reducing the image to a set of derived parameters that are can be used to reconstruct the image within a specified degree of tolerance;

inputting the derived parameters into a classification means; and

determining the classification of the image based on the output of the classification means.

63. The method of claim 62, wherein the classification means is a neural network.

64. The method of claim 62, wherein the means of reducing the image to a set of derived parameters comprises:

defining a mathematical function containing a plurality of parameters representative of the image; and

optimizing the parameters of the mathematical function using a numerical technique that minimizes the error between the image and a reconstruction of the image using the mathematical function.

65. The method of claim 62, wherein the means of reducing the image to a set of derived parameters comprises:

inputting the image to a trained neural network, wherein the inputs to the network represent the image, the network comprises a hidden layer where the number of hidden elements is smaller than the number of inputs to the network, and the outputs of the network represent the reconstruction of the image; and

setting the derived parameters to the output values of the trained neural network.

66. The method of claim 62, wherein the means of reducing the image to a set of derived parameters comprises:

defining a neural network wherein the inputs to the network are the coordinates of a point in the image, a hidden layer contains a plurality of elements, and the output of the network represents the reconstruction of the associated point in the image;

training the neural network so that the error between the network output and the image are minimized for all points in the image; and

setting the derived parameters to the weights of the hidden layer of the trained neural network.

67. The method of claim 62, wherein the processing of data process, comprises:

(i) reducing the raw reflectance data;

(ii) plotting the reduced data to generate a second image of the data;

(iii) expressing the second image as a polynomial mathematical function to generate parameters that define this image; and

(iv) comparing the parameters to parameters generated from a reference sample, whereby a positive, negative or quantitative result is obtained.

68. The method of claim 62, wherein the data is processed by a process, comprising:

(i) optionally correcting the reflectance readings to correct for light leakage;

(ii) reducing the raw reflectance data using a ratiometric formula;

(iii) generating an second image of the test data by plotting the reduced data;

(iv) expressing the second image as a polynomial mathematical function, and generating parameters that define the image;

(v) comparing the scanned image and second image by solving the linear regression through the curves;

(vi) validating the parameters obtained from the curve-fitting results to obtain a validated result; and

(vii) classifying the validated result as positive or negative by comparing peak heights of a clinical sample to reference samples.

69. The method of claim 68, wherein the data is processing process, further comprises:

(viii) inputting the validated result into a decision-support system to generate a medical diagnosis or risk assessment.

70. The method of claim 62, wherein the data is processing process, further comprises:

(viii) inputting the result into a decision-support system to generate a medical diagnosis or risk assessment.

71. The system of claim 3, wherein the test strip further comprises a symbology.

72. The system of claim 71, wherein the symbology comprises information associated with the performance or results of the assay.

73. The system of claim 71, wherein the symbology is a bar code.

74. The system of claim 71, comprising further comprising a reader for reading the symbology.

75. The system of claim 71, wherein the reader for analyzing data from the test is also adapted to read the symbology on the test device.

76. The system of claim 71, further comprising software for decoding the symbology.

77. The reader of claim 29, wherein the assay is an immunoassay.

78. The reader of claim 29, that is adapted for reading an assay test strip and a symbology that is imprinted, engraved or affixed on the test strip.

79. The method of claim 37, wherein the surface comprises an immunoassay test strip.

80. The method of claim 40, wherein the assay strip comprises a symbology that is associated with data resulting to the assay, results thereof reagents or test sample, and the method further comprises reading the symbology.

81. The system of claim 75, wherein the adaptation permits the reader to adjust to its height relative to the test device.

82. A reflectance reader, comprising:

a reader head comprising:

a reader head body;

two light emitting diodes;

a first fiberoptic bundle optically coupled to the first light emitting diode;

a second fiberoptic bundle optically coupled to the second light emitting diode;

a photodetector;

a third fiberoptic bundle optically coupled to the light photodetector;

an aperture in the reader head body;

a plurality of fiberoptic conductor ends arranged in a sigmoidal distribution in the aperture, a first portion of the fiberoptic conductor ends being of fiberoptic conductors of the first fiberoptic bundle, and a second portion of the fiberoptic conductor ends being of fiberoptic conductors of the second fiberoptic bundle.

83. The reader of claim 82 that is adapted for reading a symbology on a test device inserted into the reader.

84. The reader of claim 83, wherein the height of the reader head relative to the test device is adjustable.

85. The reader of claim 83, further comprising software for decoding the symbology.

86. The reader of claim 83, wherein the symbology is a bar code.

87. A method of developing and using a control for any assay system, comprising:

analyzing a plurality of test runs of the assay and obtaining results of the test runs;

classifying the results by type of result; and

identifying a pattern representative of or predictive of each type of result.

88. The method of claim 87, further comprising,

performing the test, and identifying the pattern contained within the results; and

classifying the test results based upon the identified pattern.

89. The method of claim 87, wherein the test results are classified as invalid based upon deviation from the identified pattern.

90. The method of any of claims 87-90, wherein classification is effected by pattern recognition means.

91. The method of any of claims 87-90, wherein the pattern recognition means is a curve fitting procedure or is a neural network.

91. The method of claim 87, wherein the pattern is curve

91. The method of claim 87, comprising:

fitting the data obtained from the assay to a predetermined mathematical function based on expected results for the assay; and

accepting or rejecting the data based on the quality of the fit between actual and expected data.

92. The method of claim 62, wherein step (b), the processing of data process, comprises:

(i) reducing the raw reflectance data;

(ii) plotting the reduced data to generate a second image of the data;

(iii) expressing the second image as a polynomial mathematical function to generate parameters that define this image;

(iv) validating the parameters obtained, wherein validation is accomplished by fitting the data obtained from the assay to a predetermined mathematical function based on expected results for the particular assay; and accepting or rejecting the data based on the quality of the fit between actual and expected data; and

(v) comparing the parameters to parameters generated from a reference sample, whereby a positive, negative or quantitative result is obtained.

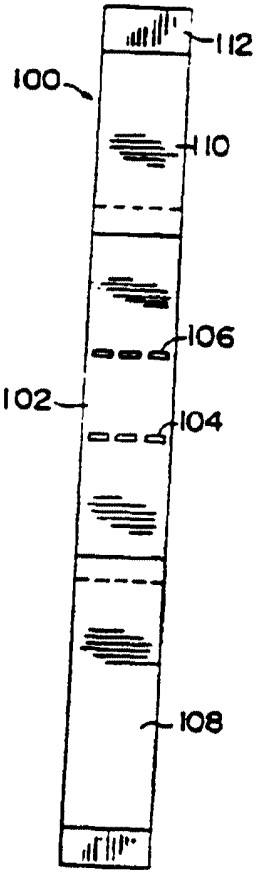


FIG. 1A

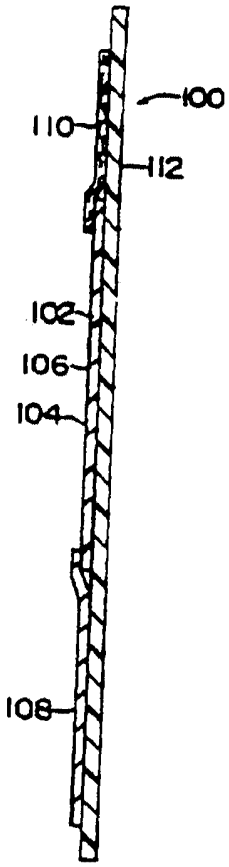


FIG. 1B

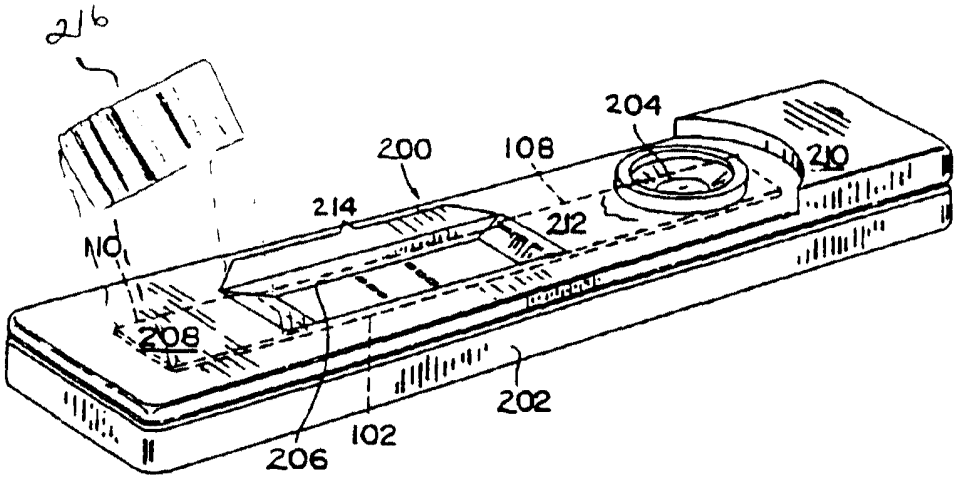

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FIG. 2A

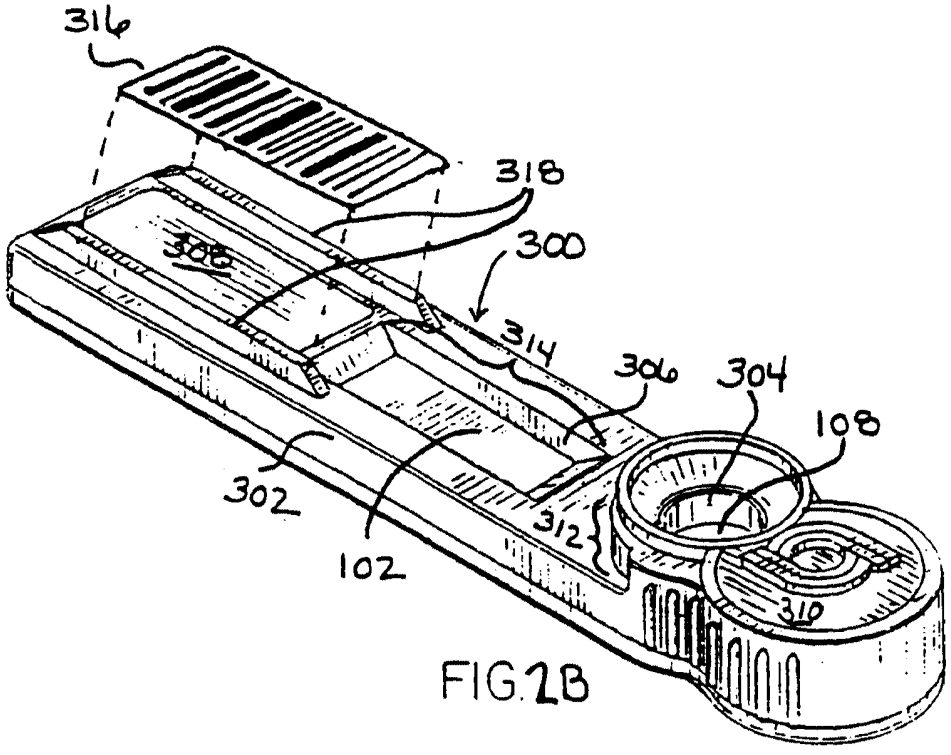

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FIG. 2B

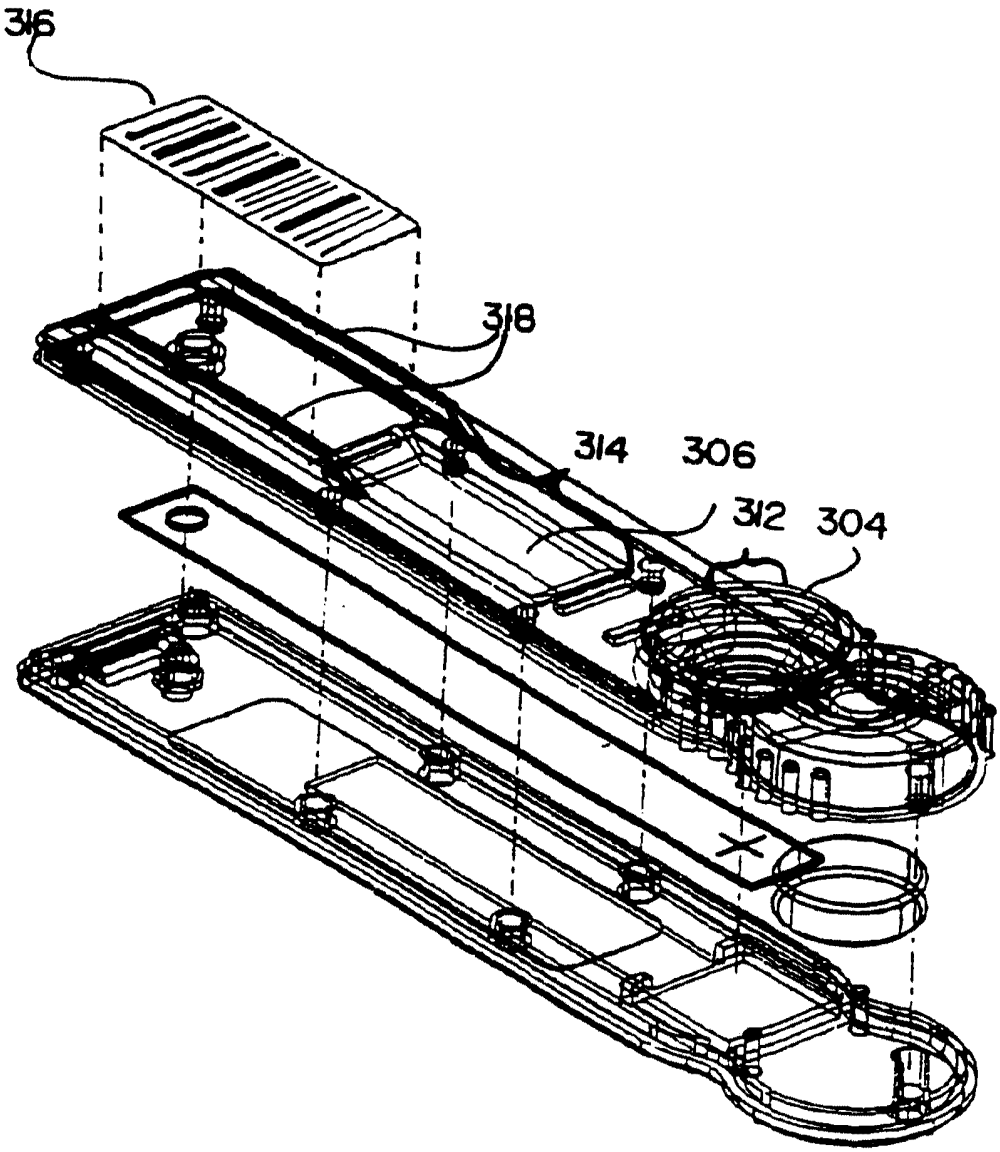


FIG. 3

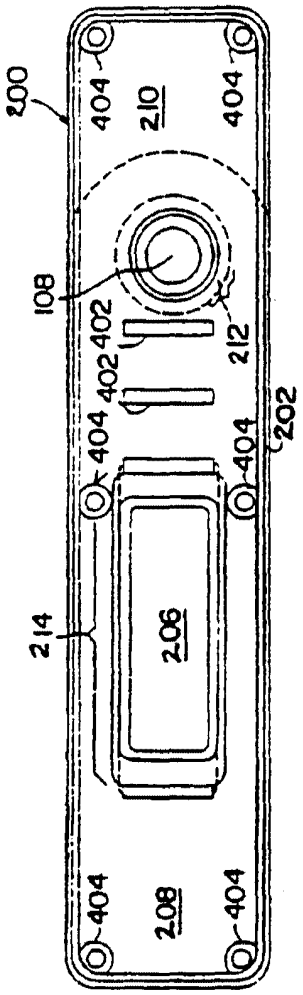

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FIG. 4

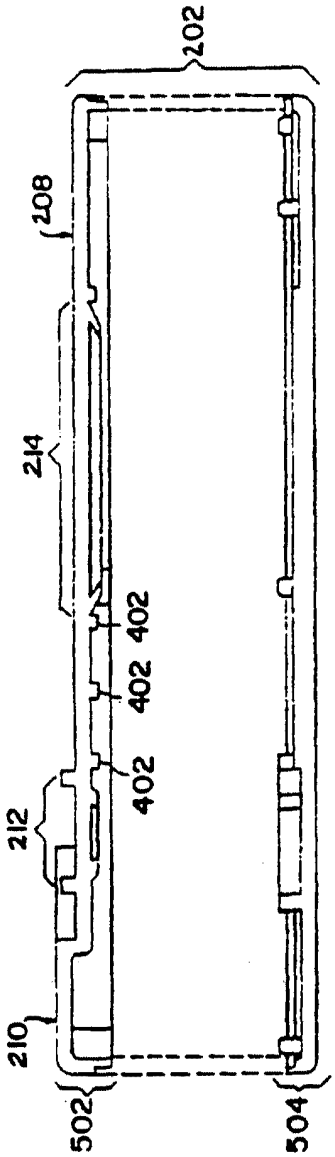

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FIG. 5

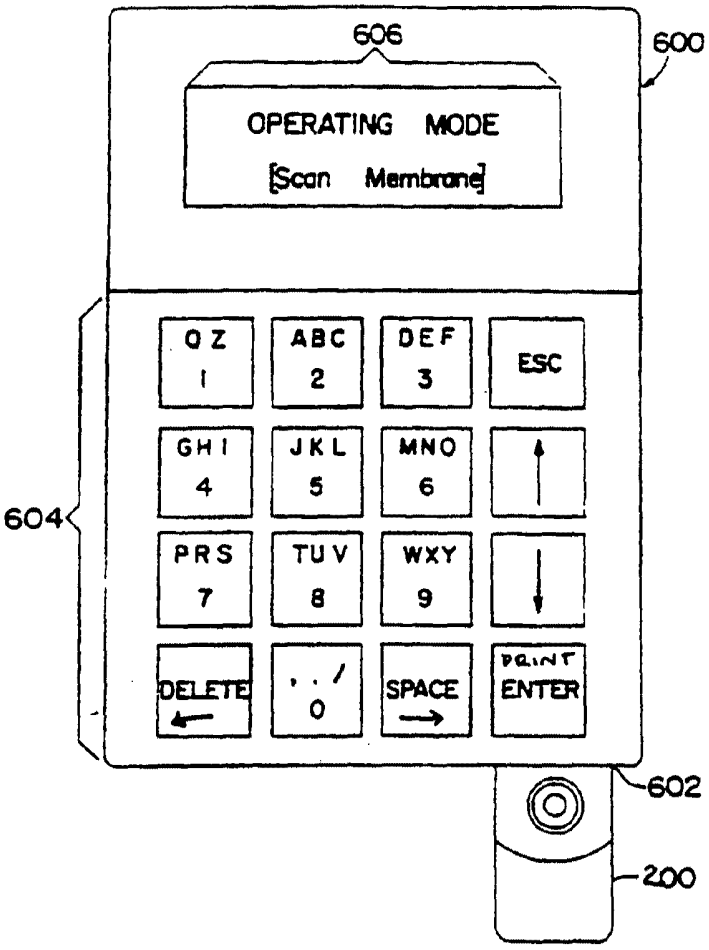

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FIG. 6

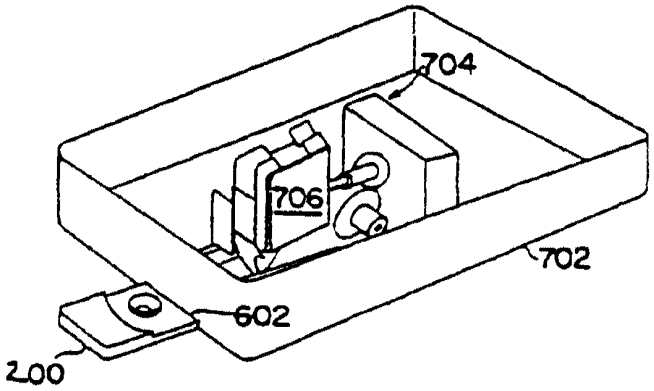


FIG. 7

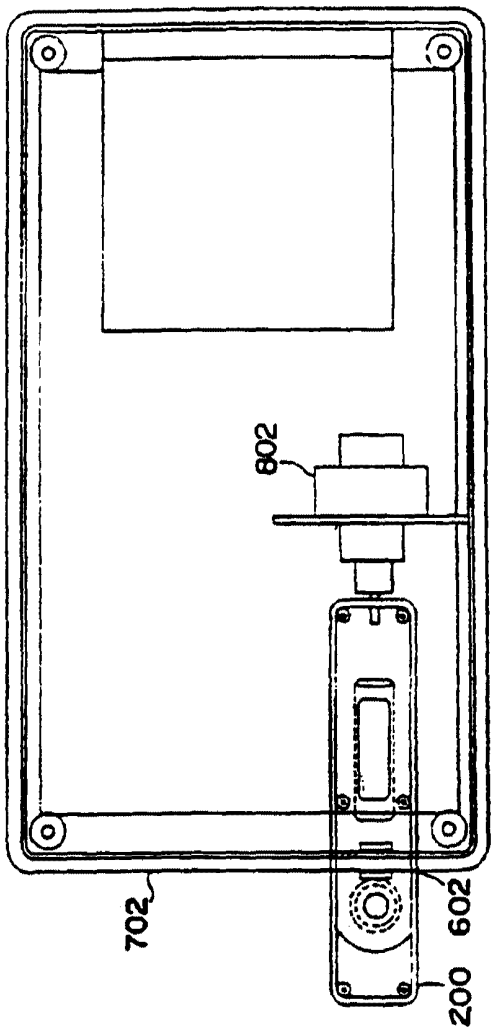


FIG. 8

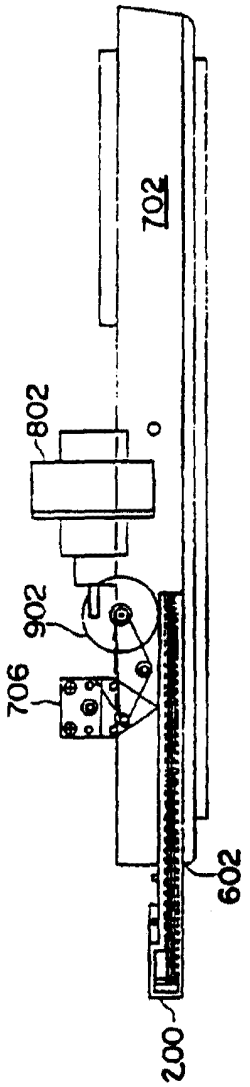

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FIG. 9

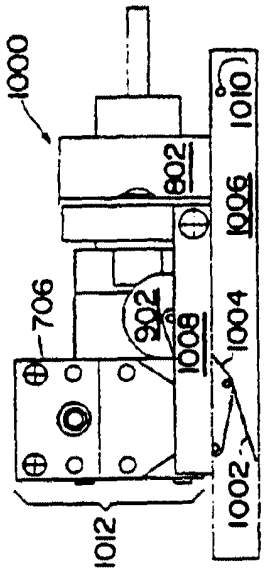

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FIG. 10

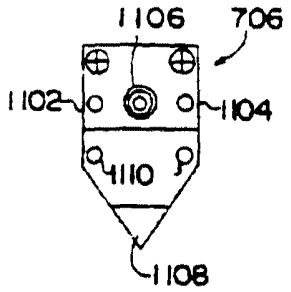


FIG. 11

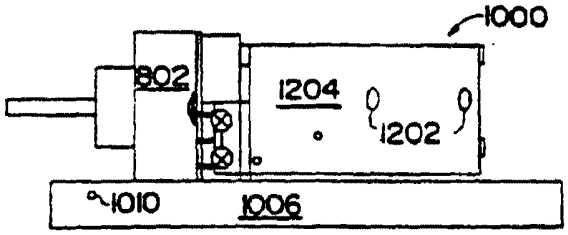


FIG. 12

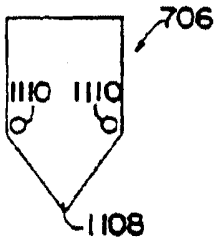


FIG. 13

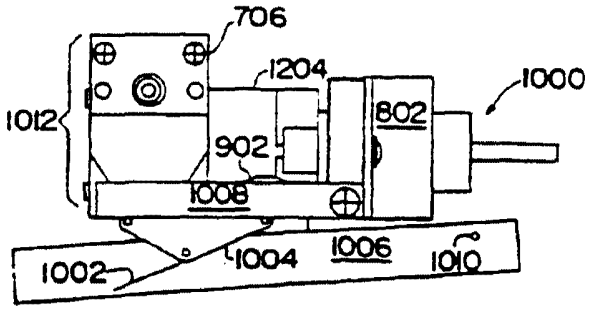


FIG.14

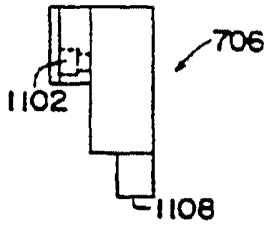


FIG.15

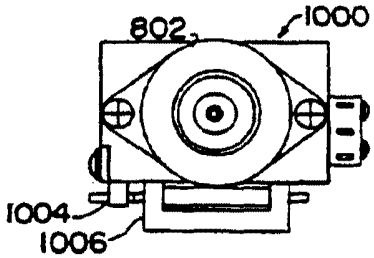


FIG.16

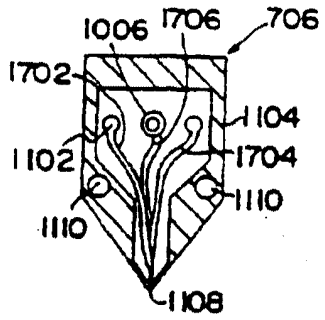

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FIG.17

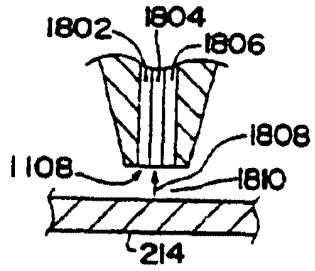

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FIG.18

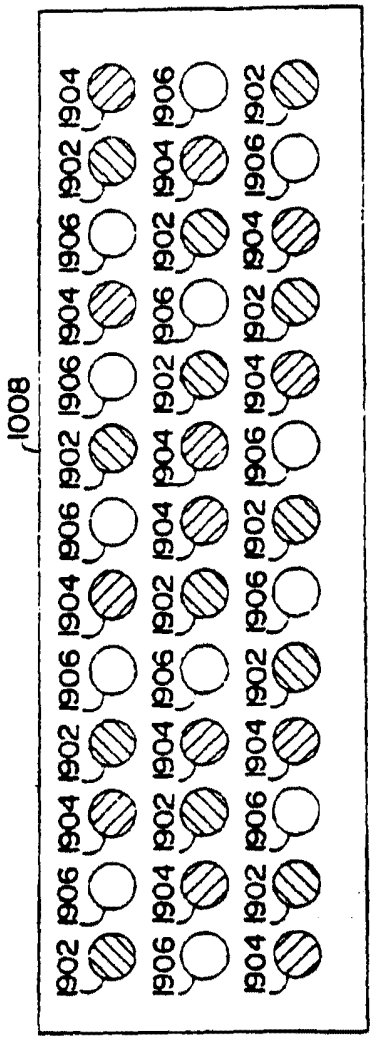

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FIG. 19

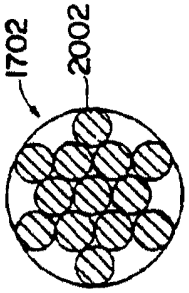

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FIG. 20

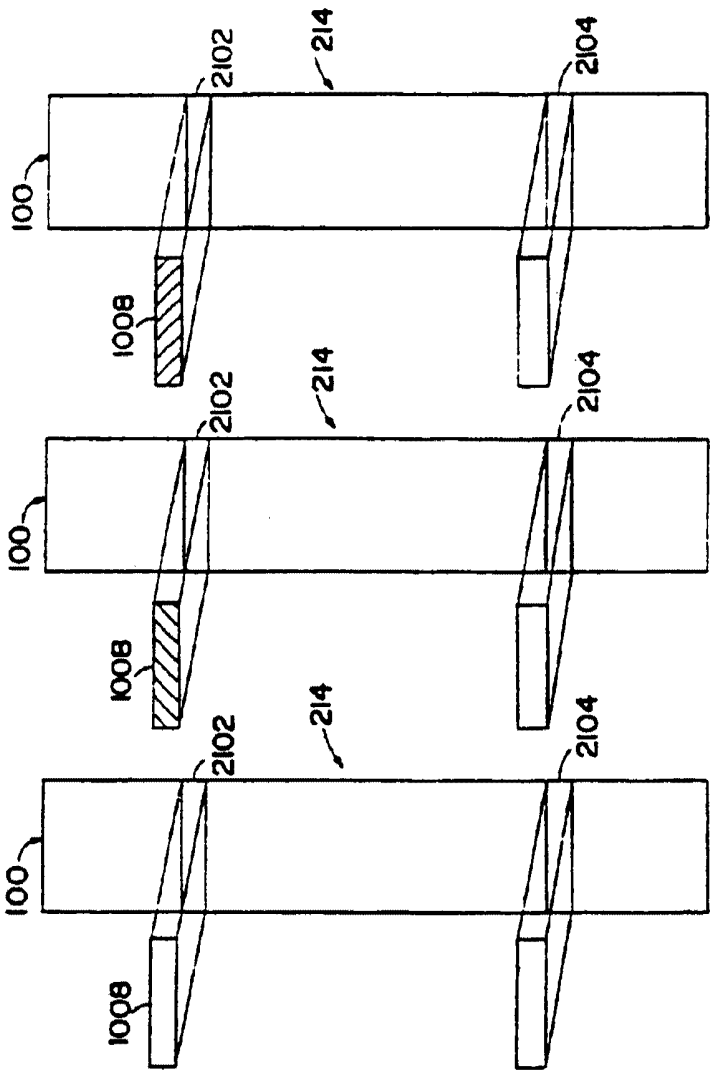


FIG.23

FIG.22

FIG.21

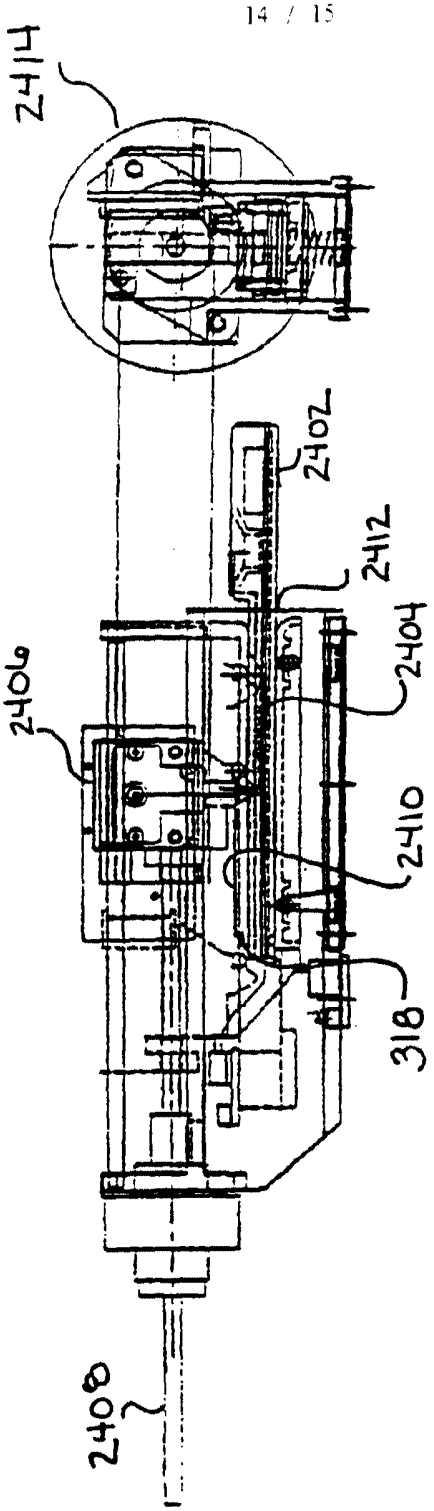


FIG. 24



FIG. 25

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/02282

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G06F19/00 G01N21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G06F G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	WO 94 27490 A (BASE TEN SYSTEMS INC) 8 December 1994 (1994-12-08) page 1, line 1 - page 7, line 25	1 2-4 87
X Y A	EP 0 644 414 A (HITACHI LTD) 22 March 1995 (1995-03-22) column 3, line 22 - column 5, line 56	57,58 19,62 54
Y A	US 5 304 468 A (PHILLIPS ROGER ET AL) 19 April 1994 (1994-04-19) column 3, line 48 - column 11, line 32; figures 1-4	2-4,19, 62 28,44, 54,82,87
A	EP 0 387 630 A (MILES INC) 19 September 1990 (1990-09-19) page 4, line 30 - page 5, line 7	87

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 July 1999	13/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schenkels, P

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/02282

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of the relevant passages	Relevant to claim No
A	US 5 321 492 A (DETWILER PAUL S ET AL) 14 June 1994 (1994-06-14) column 1, line 13 - column 3, line 9 -----	1,28,44, 54,82

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intc onal Application No
PCT/US 99/02282

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G09F 3/02 — A1

(11) International Publication Number: WO 99/36901

(43) International Publication Date: 22 July 1999 (22.07.99)

(21) International Application Number: PCT/US98/10300

(22) International Filing Date: 20 May 1998 (20.05.98)

(30) Priority Data:
09/007,531 15 January 1998 (15.01.98) US

(71) Applicant: MINNESOTA MINING AND MANUFACTURING COMPANY [US/US]; 3M Center, P.O. Box 33427, Saint Paul, MN 55133-3427 (US).

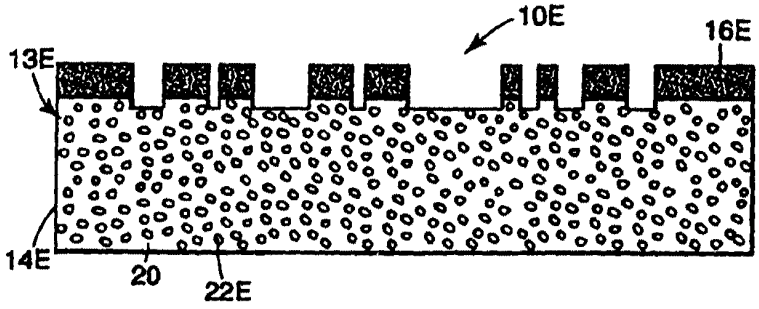
(72) Inventors: MOH, Kyung, H.; P.O. Box 33427, Saint Paul, MN 55133-3427 (US). LOCAVE, Daniel; P.O. Box 33427, Saint Paul, MN 55133-3427 (US). SUEOSS, Bernardus, M.; P.O. Box 33427, Saint Paul, MN 55133-3427 (US).

(74) Agents: FEULNER, Gregory, J. et al.; Minnesota Mining and Manufacturing Company, Office of Intellectual Property Counsel, P.O. Box 33427, Saint Paul, MN 55133-3427 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MULTILAYER, TEMPERATURE RESISTANT, COMPOSITE LABEL



(57) Abstract

A composite material suitable for labeling a substrate. The composite material, which is preferably a ceramic composite, comprises a fired ceramic body and a layer thereon. The fired ceramic body includes a base layer that comprising a glassy phase and a refractory phase, the glassy phase being capable of wetting a substrate at an application temperature. There is sufficient color contrast between the top layer and the fired ceramic body such that a code pattern (e.g., a bar code) present (or formed) is optically discernible. Methods of making and using the same are also taught.

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MULTILAYER, TEMPERATURE RESISTANT, COMPOSITE LABEL

FIELD OF THE INVENTION

The present invention relates to composite labels useable at high temperatures (e.g., temperatures above about 400°C). More particularly, the present invention relates to high temperature composite labels in which the label body includes a glassy phase and a refractory phase, and in which an optically discernible code pattern is, or can be, formed in a label top layer.

BACKGROUND OF THE INVENTION

Parts and products can be tracked during manufacturing and marketing operations in order to facilitate commodity distribution, customizing product features, process control, stock control, sales control, quality control, and the like. Such tracking requires some means for marking or labeling parts and products so that such parts and products can be readily and accurately identified during tracking. For example, according to one approach, a label bearing identification information in human readable form (e.g., alphanumeric information) or machine readable form (e.g., bar code information), or both, is attached to parts running on a production line. The label is read at one or more process stations so that specific operation steps can be carried out according to work schedules corresponding to the identification information.

In some industries, printed paper labels are used for tracking purposes. However, for other industries, (e.g., steel making industries and cathode ray tube industries) production lines operate at temperatures well above ambient conditions (e.g., above 250°C, or even above 400°C). For example, the manufacture of a glass cathode ray tube typically involves temperatures in the range from about 400°C to about 1000°C. Paper labels do not survive at these temperatures. Consequently, labels that can be easily attached to parts and maintain their integrity at such temperatures are needed.

Many parts have nonplanar surfaces to which a label is attached in order for such parts to be tracked. For example, a cathode ray tube includes a glass cone component and a glass faceplate component, both of which have curved, nonplanar surfaces. Consequently, not only is there a need for temperature resistant labels, but there is also a need for temperature resistant labels which are conformable to nonplanar substrates at application temperatures.

Another concern relates to the durability of labels. It is most desirable if a label is durable enough to survive the entire production process without requiring interim replacement. Unfortunately, many of the previously known labels adapted for high temperatures become disfigured and/or unreadable too easily and must be replaced several times during production in order to preserve tracking capabilities. Other labels, although durable, have a tendency to delaminate and fall off of their substrates. Consequently, labels with both excellent durability and tenacious bonding characteristics would be desirable.

As a practical matter, production yields seldom reach or are maintained at 100%. For example, parts can break, fail to meet specifications, or suffer from other defects that necessitate discarding or recycling of the parts. Recycling is often more desirable than discarding bad parts, particularly when the raw materials incorporated into the parts are relatively expensive. However, before recycled materials can be returned to the production line, contaminants are preferably removed to maintain the quality of parts formed from recycled material. Unfortunately, many of the previously known labels would contaminate the recycled supply and must be removed. Labels comprising heavy metal atoms such as Pb, Hg, As, Co and Cr(VI), whether present in pure form or in the form of oxides, are of particular concern due to the toxicity associated with such materials. Oxides of heavy metals such as Pb, Cd, Hg, and/or As are known to be incorporated into glassy phases as one approach for achieving low softening points (e.g., softening points below about 350°C). Oxides of heavy metals such as Co and Cr are known to be used for providing black color. Consequently, articles having a label comprising one or more these heavy metals are typically recycled by delabeling and separating the less desirable heavy metals containing labels from the articles. These steps can be costly and/or extremely difficult, particularly if the

bond between the article and the label is especially tenacious. In addition, heavy metals may be dispersed in air when the labels containing such metals are heated to high temperatures. Accordingly, it would be desirable to provide a label substantially free of these heavy metal atoms.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a composite material (typically a ceramic composite) suitable for labeling a substrate, comprising:

(a) a fired ceramic body comprising a base layer, the base layer including:

(i) a first glassy phase;

(ii) a first refractory phase (preferably a ceramic material such as particulate ceramic material) interspersed with the first glassy phase,

the first glassy phase being capable of wetting a substrate at an application temperature; and

(b) a top layer provided on the fired ceramic body,

wherein there is sufficient color contrast between the top layer and the fired ceramic body such that a code pattern (preferably formed by selectively removing the top layer from portions of the composite layer) is optically discernible. Optionally, a label according to the present invention may further comprise a translucent (including transparent or clear) cover (e.g., a protective layer or a coating) over the top layer. A protective layer (e.g., a glass layer) is sufficiently translucent to allow a bar code reader, human eye, or other detector means to view or read the underlying code pattern. Optionally, the translucent layer(s) is a fugitive material.

Embodiments of labels according to the present invention are capable of retaining not only their dimensional integrity, but also the integrity of information incorporated into the labels, especially when the label is exposed to high temperatures (e.g., temperatures above 250 °C, preferably 400 °C to 1000 °C). In another aspect, labels according to the present invention can be conveniently applied to a wide range of substrates, including metal, glass, ceramics, and the like, which are intended to be used at relatively high temperatures.

Labels according to the present invention comprise a top layer of one color overlying a layer of a contrasting color. When portions of the top layer are selectively removed to expose portions of the underlying layer, the color contrast between layers allows the resultant pattern formed from the top layer to be optically discernible. This allows information to be incorporated into the label by selectively patterning or removing the top layer to form one or more symbols of a code pattern either as a negative or a positive image, as desired. Such a code pattern, in turn, allows a labeled substrate to be easily identified and tracked during production. The code pattern(s) can be formed in the label either before and/or after the label is attached to a corresponding substrate. Information can be incorporated into the label one or more times during the service life of the label as well. This is particularly advantageous for production operations in which updating label information during the course of production is desired.

The code pattern incorporated into the label may be human readable, machine readable, combinations of these, and the like. In preferred embodiments, the code pattern includes human readable alphanumeric characters, machine readable bar code characters, or combinations thereof. Bar code patterns, in particular, can be easily and rapidly formed in labels of the present invention using, for example, well known laser ablating techniques.

Because of the color contrast and multilayer construction of labels according to the present invention, code patterns can be formed by abrasion, etching or abrading techniques in which substantially only portions of the top layer are removed. In other words, material can be selectively removed from the label only to a depth substantially corresponding to the thickness of the top layer. Very little if any of the underlying label layers need be subjected to such removal, and the substrate itself remains unaltered. Advantageously, therefore, the underlying layers of the label can further serve as a protective barrier between the patterning applied to the top layer and the substrate below. In this way, the substrate can be protected from damage that might otherwise occur if such patterning techniques were to be applied directly to the substrate itself. In another aspect, the code pattern can be formed directly, for example, by depositing the pattern to the top layer using a surface printing technique such as ink jet printer with a ceramic oxide

containing ink or depositing the composition through a mask.

Labels according to the present invention include a structural base layer incorporating both a glassy phase and a refractory phase. This composite structure makes it easy to firmly attach the labels to a wide variety of substrates at an application temperature at which the glassy phase liquifies and wets the substrate surface. Because the glassy phase itself bonds to the substrate, no other adhesives are required to achieve label attachment. When the glassy phase is liquified at the application temperature, the refractory phase structurally and dimensionally supports the base layer. Simultaneously, the base layer can be pliable due to the presence of the melted glassy phase, thereby allowing the label to conform to nonplanar substrates, while the presence of the reinforcing refractory phase allows the label to retain its dimensional and structural integrity as the label is flexed, bent, or otherwise shaped to conform to such a substrate.

In applications in which the substrate is formed from a glass and/or a crystalline ceramic, the coefficient of thermal expansion of a label of the present invention can generally be matched as closely to the coefficient of thermal expansion of the substrate as desired. Matching the coefficients of thermal expansion of the substrate and label offers numerous advantages. For example, such matching enhances the strength of the bond between the label base layer and the substrate. As a further advantage, such matching minimizes thermally induced stresses between the substrate and label as temperatures change. If the coefficients of thermal expansion are not matched well, thermal induced stresses can damage the label and/or the substrate.

In preferred embodiments, labels according to the present invention preferably include substantially no heavy metal atoms selected from Pb, Cd, As, Hg, Co, and Cr(VI). Such heavy metal atoms generally pose environmental and/or health hazard so that avoiding the use of such materials is highly desirable. Providing labels that are free of these kinds of heavy metal atoms also facilitates recycling, because both the label and the substrate can be processed for recycling together without having to remove the label. If the label were to incorporate these kinds of heavy metal atoms, the label might be an undesirable contaminant that would have to be removed from the substrate in the event recycling is desired.

This would involve extra expense and processing steps. Avoiding such heavy metal atoms as label constituents is particularly advantageous for industrial applications such as the manufacture of cathode ray tubes, where breakage is known to occur and recycling is regularly practiced.

Another aspect of the present invention relates to a labeled cathode ray tube, or components thereof (e.g., faceplates and funnels). The labeled cathode ray tube comprises a cathode ray tube glass surface and at least one fired composite label according to the present invention attached to the cathode ray tube glass surface.

In yet another aspect, the present invention provides a method of applying a label (including a blank label (i.e., a label without the code pattern)) according to the present invention to a substrate, the method comprising the steps of;

(a) providing and/or heating a substrate (e.g., a ceramic material) at and/or to an application temperature; and

(b) attaching the label to the substrate such that the first glassy phase of the label bonds to the substrate.

Optionally, the label is heated to and/or is at the application temperature rather than the substrate, or both the substrate and the label are heated to and/or are at the application temperature.

In this application:

"Softening point" refers to the temperature at which the viscous flow of a glassy composition counteracts thermal expansion as determined according to ASTM test method E 1545-95A (December, 1995) entitled "Standard Test Method for Assignment of Glass Transition Temperature by Thermomechanical Analysis". Such a temperature generally corresponds to the maximum point on a thermal expansion curve. In this method, the softening point is referred to as the "dilatometric" softening point.

"Ceramic" refers to an inorganic, nonmetallic material, such as metal oxides, metal nitrides, and metal oxynitrides, preferably consolidated by the action of heat.

"Fired" refers to densification or consolidation by action of heat.

"Refractory" refers to a material that maintains its structural integrity at

temperatures at least up to about 1000 °C.

"Glassy" refers to an amorphous inorganic oxide material having a softening point above which the material melts or softens. Amorphous materials typically have a diffuse x-ray diffraction pattern without definite lines that might otherwise indicate the presence of a crystalline phase.

BRIEF DESCRIPTION OF THE DRAWING

The above mentioned and other advantages of the present invention, and the manner of attaining them, will become more apparent and the invention itself will be better understood by reference to the following description of the embodiments of the invention taken in conjunction with the accompanying drawings, wherein:

FIG. 1 is a schematic cross-sectional view of a label according to the present invention attached to a substrate, wherein the label has a dark colored base layer and a light colored top layer;

FIG. 2A is a perspective view of the label of FIG. 1, wherein a negative code pattern has been formed by removing portions of the top layer;

FIG. 2B is a perspective view of the label of FIG. 1, wherein a positive code pattern has been formed by removing portions of the top layer;

FIG. 3 is a schematic cross-sectional view of another label according to the present invention attached to a substrate, wherein the label has a light colored base layer and a dark colored top layer;

FIG. 4 is a schematic cross-sectional view of another label according to the invention wherein the base layer is light colored and the top layer is a metal or metal alloy;

FIG. 5 is a schematic cross-sectional view of the label of FIG. 4, wherein a code pattern is incorporated into the top layer; and

FIG. 6 is a schematic cross-sectional view of another label according to the present invention, wherein the label body incorporates an intermediate layer interposed between the base layer and the top layer.

DETAILED DESCRIPTION

The embodiments of the present invention described below are not intended to be exhaustive or to limit the invention to the precise forms disclosed in the

following detailed description. Rather the embodiments are chosen and described so that others skilled in the art may appreciate and understand the principles and practices of the present invention.

FIG. 1 shows one preferred embodiment of a fired composite ceramic label according to the present invention. Fired composite ceramic label according to the present invention 10 is attached to substrate 12. The structures of label 10 and substrate 12 have not been drawn to scale, but rather have been represented schematically for purposes of illustration. Substrate 12 may be any of a wide variety of substrates for which labeling with a ceramic-based label is desired. Label 10 is particularly useful, however, in high temperature industrial labeling applications involving elevated temperatures of at least 250°C, preferably at least 400°C, more preferably in the range from 400°C to 1800°C, and even more preferably in the range from 400°C to about 1000°C, in which substrate 12 is made of a metal, a metal alloy, a glass, a crystalline ceramic, other refractory materials, combinations of these, and the like. Representative examples of just a few of the many kinds of substrates that advantageously can be labeled in accordance with the principles of the present invention include liquid crystal displays (LCD's), flat glass, curved glass, ceramic whiteware, wine bottles, nuclear waste tanks, glass surfaces of cathode ray tube components (e.g., cones and faceplates), motor vehicle components, aircraft engine components, rocketry components, and the like. As shown, substrate 12 is curved, although this is not required. Label 10 can be attached to other kinds of substrates which are flat, cone-shaped, undulating, irregularly textured, otherwise nonplanar, or have any other desired configuration.

Optionally, a label according to the present invention may include an indicator element or material, or markings, for example, on the surface of the label or within the label composition, enabling a user to select a particular label or label type for a specific application (e.g., selecting the correct label type for a specific glass substrate). For example, the base layer, top layer, and/or other layer(s) may have colors or a certain color combination to allow for easy differentiation between labels designed for different application temperatures.

Advantageously, label 10 includes a composite composition that allows label 10 to be sufficiently pliable at a desired, elevated application temperature so

as to be conformably attachable to nonplanar substrate surfaces while still maintaining structural and dimensional integrity. Some preferred embodiments of the present invention are conformable to surfaces with compound curvature. The term "compound curvature" refers to a surface which is curved about more than one axis. For example, the surfaces of a sphere or a saddle are examples of surfaces with a compound curvature. Typically, a surface with compound curvature cannot be formed from a flat sheet without stretching at least some region of the sheet. In contrast, a cylindrical surface is a surface with simple curvature about one axis.

Label 10, as illustrated, has a two layer construction including ceramic body 13 and top layer 16 provided on ceramic body 13. Ceramic body 13 is in the form of base layer 14. Although only base layer 14 is shown as an element of fired ceramic body 13 in this embodiment, ceramic body 13 may also be formed with multiple layers. One such multiple layer, alternative embodiment will be described in more detail below in connection with FIG.6.

Still referring to FIG. 1, base layer 14 has fired, composite ceramic composition incorporating glassy phase 20. Base layer 14 also incorporates a refractory phase in the form of plurality of refractory particles 22 interspersed in glassy phase 20. Glassy phase 20 preferably has softening point characteristics effective to provide label 10 with desired physical and mechanical properties for the intended use. For example, glassy phase 20 preferably has a softening point so that glassy phase 20 is capable of wetting and thereafter fuseably bonding to substrate 12 at the desired application temperature. If the softening point is too high relative to the application temperature, it may be difficult to bond label 10 to substrate 12. If the softening point is too low relative to the desired application temperature, glassy phase 20 may flow too much during application to substrate 12, causing ceramic body 13 to lose its dimensional and/or structural integrity.

Accordingly, given a particular application temperature at which it is desired to attach label 10 to substrate 12, it is preferred that the application temperature is from about 100°C to about 300°C greater than the softening point of glassy phase 20. To illustrate this relationship, the following table shows suitable softening point ranges for glassy phase 20 at application temperatures of 550°C,

750°C, and 800°C, respectively:

Application Temperature	Corresponding Softening Point Range of Glassy Phase 20
550°C	250°C to 450°C
750°C	450°C to 650°C
800°C	500°C to 700°C

Given a desired application temperature, one skilled in the art would be able to provide a glassy phase having the desired softening point characteristics.

Similar to base layer 14, top layer 16 also preferably has fired, composite ceramic composition incorporating a glassy phase 24 and refractory phase in the form of a plurality of refractory particles 26 interspersed in glassy phase 24. Glassy phase 24 desirably has a softening point effective to allow top layer 16 to be sufficiently conformable to substrate 12 along with base layer 14 without fracturing and without experiencing undesirable levels of flow at the desired application temperature of label 10. In preferred embodiments, the softening point of glassy phase 24 preferably is greater than the softening point of glassy phase 20. More preferably, the softening point of glassy phase 24 is greater than the softening point of glassy phase 20, and the difference between such softening points is preferably in the range from 5°C to 50°C, more preferably 5°C to 20°C.

The color contrast, physical, mechanical, and thermal properties of base layer 14 and/or top layer 16 can be tailored, for example, through appropriate selection of the amount and kind of refractory particles 22 and/or 26 incorporated into either layer. For example, refractory particles 22 and 26 may be selected to provide base layer 14 and top layer 16 with first and second colors, respectively, between which there is sufficient contrast such that a code pattern (not shown in FIG. 1) formed from top layer 16 is optically discernible when the underlying base layer 14 is exposed by removing portions of the top layer 16. "Optically discernible" means that such a code pattern is human and/or machine readable using optical techniques. Representative examples of such optical techniques includes viewing and/or scanning using infrared (IR) light, visible light, ultraviolet

(UV) light, or the like. Labels that are intended to be legible in the IR and/or UV ranges may not be discernible in the visible range. As an option, the light source may be collimated or uncollimated. In preferred embodiments, one of layers 14 or 16 is black, and the other layer is white.

In the embodiment shown in FIG. 1, base layer 14 is relatively dark (e.g., black) and top layer 16 is relatively light (e.g., white). In order to provide base layer 14 with a relatively dark color, relatively dark refractory particles 22 can be incorporated into base layer 14. In order to provide top layer 16 with a relatively light color, relatively light refractory particles 26 can be incorporated into top layer 16. With this approach, selective removal of portions of relatively light top layer 16 will expose the relatively dark, underlying base layer 14. Portions of top layer 16 can be removed to form either a negative or positive code pattern. As used herein, a "negative" code pattern is a pattern in which the portions of the top layer corresponding to the code pattern characters themselves are removed from the top layer to form the code pattern. A "positive" code pattern is a pattern in which portions of the top layer around the code characters are removed so that the remaining portions of the top layer form the code pattern. As an option, a code pattern formed from the top layer may include one or more negative code pattern portions, one or more positive code pattern portions, or a combination of one or more positive code patterns and one or more negative code patterns.

FIG. 2A shows label according to the present invention 10A identical to label 10 of FIG. 1, except that portions of top layer 16A have been removed to form negative alphanumeric code pattern "12-34567". In this embodiment, only portions of top layer 16A corresponding to the alphanumeric characters themselves have been removed to expose the relatively dark, underlying base layer 14A of ceramic body 13A.

On the other hand, FIG. 2B shows label according to the present invention 10B identical to label 10 of FIG. 1, except that portions of relatively dark top layer 16B have been removed to form positive alphanumeric code pattern "12-34567". Base layer 14B of ceramic body 13B is relatively light. In this case, all portions of top layer 16B except the portions corresponding to the characters themselves have been removed. Because so much less material needs to be removed from top layer

16B to form the negative code pattern of FIG. 2A than the positive code pattern of FIG. 2B, label 10A is a preferred configuration to be used when code patterns having dark code characters are desired. In some applications, recycling considerations may be more important than the convenience of feature formation. For example, removing almost all of a highly colored layer to leave only a small amount of colored material in a positive image may be more desired in recycling schemes for which the colorant would be a contaminant.

When forming a code pattern such as the code patterns shown in FIGS. 2A and 2B, the code pattern can be formed in top layer 16 using any technique suitable for removing portions of top layer 16, including abrasion, ablating or etching techniques. In the practice of the present invention, the use of laser ablating techniques is presently preferred for forming code patterns in top layer 16. Laser ablating is well-known, economical, fast, and provides patterns with sharp, precise features. A code pattern can be formed in top layer 16 before and/or after label 10 is attached to substrate 12, as desired.

Alternatively, rather than using abrading, ablating, or etching techniques to pattern layer 16, the code pattern can be formed directly by depositing the patterned form of top layer 16 using a suitable printing technique such as ink jet printing with a ceramic oxide containing ink or depositing the composition through a mask. Ink jet printing techniques are described, for example, in U.S. Pat. No. 5,407,474 (Airey et al.).

The code pattern can be formed in the top layer, for example, by the above techniques at various temperatures (e.g., ambient and/or high temperatures (e.g., temperatures above 250°C, preferably 400°C to 1000°C)).

In another aspect, some techniques, such as laser ablating, may be used to remove various thicknesses of the top layer of the label, to allow, for example, different levels of contrast on the same label. For example, for a top layer having some translucency, the degree of contrast in a code pattern can be adjusted or varied by varying the thickness of the top layer removed. The greatest contrast being obtained for a given label when the total thickness of the top layer is removed (i.e., when the underlying layer is exposed).

FIG. 3 shows an alternative embodiment of label according to the present invention 10C which is the same as label 10 of FIG. 1, except that base layer 14C has a relatively light color provided by relatively light colored refractory particles 22C, and top layer 16C has a relatively dark color provided by relatively dark refractory particles 24C.

In order to provide a layer with a dark color, the use of substantially black refractory particles is preferred. Representative suitable black refractory particles may be selected, for example, from particles of silicon carbide, titanium carbide, zirconium carbide, graphite, combinations of these, and the like. The dark color may also be provided by inclusion of dark-colored, nonrefractory particles such as carbon black, MnO_2, combinations of these, and the like. In order to provide a layer with a light color, the use of substantially white refractory particles is preferred. Representative examples of suitable white refractory particles may be selected, for example, from alumina, zirconia, zircon, mullite, titania, combinations of these, and the like. Other materials for the (light-colored or dark-colored) refractory particles may include those made of metal (e.g., platinum, rhodium, and the like) and/or a cermets.

Referring again primarily to FIG. 1, refractory particles 22 also contribute to preferred dimensional and structural integrity characteristics of base layer 14. For example, if too few refractory particles 22 are included in base layer 14, glassy phase 20 may flow too much at the application temperature. Advantageously, a sufficient amount of refractory particles 22 are incorporated into base layer 14 to block such undesirable flow of glassy phase 20, thereby helping to maintain the structural and dimensional integrity of base layer 14. Additionally, if too few refractory particles 22 are included in base layer, the resultant base layer 14 may be too brittle, and/or base layer 14 may be too sticky at the application temperature for convenient handling prior to attachment of base layer 14 to substrate 12. On the other hand, if base layer 14 incorporates too many refractory particles 22, base layer 14 may not allow sufficient flow of the glassy phase to promote good bonding, or base layer 14 may be so rigid as to lack a desired amount of pliable conformability. Generally, balancing these concerns, a preferred base layer 14 of the present invention incorporates a sufficient amount of refractory particles 22

such that base layer 16 is pliably conformable at the desired application temperature if necessary to conform to a surface of the substrate (e.g., a flat label needs to be pliably conformable at the desired application temperature to conform to a substrate having a curved surface). More preferably, base layer 14 incorporates enough refractory particles 22 such that base layer 14 includes 10 to 60, preferably 30 to 50, volume percent of glassy phase 20 and 40 to 90, preferably 50 to 70, volume percent of refractory particles 22.

Because top layer 16 is fully supported by the dimensionally stable, yet conformable base layer 14, refractory particles 26 need not serve a primary structural and dimensional function as do refractory particles 22 in base layer 14. Refractory particles 26, however, do provide a convenient means for coloring top layer 16 to ensure that a code pattern formed from top layer 16 is optically discernible from base layer 14. Accordingly, refractory particles 26 may be incorporated into top layer 16 in lesser amounts so long as enough particles 26 are used to provide top layer 16 with desirable color contrast characteristics. To this end, preferred top layers 16 of the present invention incorporate 5 to 50 parts by weight, more preferably 10 to 40 parts by weight of refractory particles 26 per 100 parts by weight of glassy phase 24.

In addition to contributing to color contrast, mechanical strength, dimensional and structural stability, and conformability, refractory particles 22 and 26 provide numerous other benefits as well. For example, using appropriate amounts of refractory particles 22 or 26, as the case may be, also helps provide each of base layer 14 and top layer 16, respectively, with a desired coefficient of thermal expansion. For example, it is preferred that base layer 14, top layer 16, and substrate 12 have similar coefficients of thermal expansion to minimize thermal stresses when substrate 12 bearing label 10 is subjected to temperature changes. This is particularly beneficial when hot substrates are cooled to room temperature from temperatures above 400°C. If the coefficients of thermal expansion of the layers 14 and 16 and substrate 12 are too far apart, then thermal stresses could cause label 10 and/or substrate 12 to warp, crack, or otherwise suffer damage.

Accordingly, it is generally desirable if the ratio of the coefficients of thermal expansion of each of base layer 14 and top layer 16 to that of substrate 12

be in the range of 0.8 to 1.2, preferably 0.9 to 1.1 over a temperature range extending from about room temperature to about the desired application temperature. In the practice of the present invention, the coefficient of thermal expansion of a material is measured by ASTM E831, "Standard Test Method for Linear Thermal Expansion of Solid Materials by Thermomechanical Analysis" (September 1993). As an example, cathode ray tube glass typically has a coefficient of thermal expansion in the range from 8 x 10^{-6}/°C to12 x 10^{-6}/°C over a temperature range extending from 25°C to 300°C. For cathode ray tube glass having a coefficient of thermal expansion of about 8 x 10^{-6}/C, it is preferred that each of base layer 14 and top layer 16 independently have a coefficient of thermal expansion in the range from 6.4 x 10^{-6}/°C to about 9.6 x 10^{-6}/°C. Similarly, for cathode ray tube glass having a coefficient of thermal expansion of about 12 x 10^{-6}/°C, it is preferred that each of base layer 14 and top layer 16 independently have a coefficient of thermal expansion in the range from 9.6 x 10^{-6}/°C to 14 x 10^{-6}/°C.

In addition, refractory particles 22 and 26 may help minimize carbon trapping during firing, which can contribute to an off white color and reduce the contrast ratio of the formed image. In white-colored layers, refractory particles 22 and 26 with high refractive index may be used to enhance the white color.

Refractory particles 22 and 26 may each be provided with a wide range of particle sizes and shapes with beneficial results. Excessive porosity is generally undesirable, because layers with too much porosity tend to be mechanically weak. On the other hand, if the particles are too big, then the resultant layer may be too rough and/or mechanically weak. Generally, in preferred embodiments according to the invention, refractory particles 22 and 26 independently have an average particle size in the range from 2 micrometers to about 10 micrometers. In terms of shape, refractory particles 22 and/or 26 independently may be spherical, ellipsoid, platelet-shaped, acicular, or the like. However, acicular-shaped particles typically provide layers with the better impact resistance as compared to layers incorporating particles of other shapes.

Typically, refractory particles 22 and/or 26 have a normal particle size distribution. As an option, however, refractory particles 22 and/or 26 having

bimodal or other multimodal size distributions could be used, if desired. For example, the appropriate selection of a combination of two or more particle size distributions may help refractory particles 22 and/or 26 to further viscosify glassy phases 20 and/or 24 to prevent excessive flow at the application temperature as compared to particles having only a normal size distribution. Viscosification may also help glassy phase 20 more effectively fuseably bond to substrate 12. In combination with blockage of glassy flow during firing and/or during application, such viscosification, or thickening, of glassy phase 20 may further help maintain the structural and dimensional integrity of base layer 14 without significantly limiting conformability. Accordingly, in applications in which it is desirable to further viscosify glassy phase 20 and/or 24, at least a portion, preferably 5 to 10 weight percent, of refractory particles 22 and/or 26 may include an amount of finely divided refractory particles (i.e., refractory particles have an average particle size of less than 1 micrometer, preferably 0.5 to 1 micrometer) effective to substantially prevent undesired flow of glassy phase 20 and/or 24.

In the preferred embodiments, the refractory particles of the base layer provide a loose framework which supports and determines the shape of the base layer. The glass phase fills the void volume within the refractory framework. If the volume of the glass phase is significantly lower than the available void volume, the glass may not flow sufficiently well from the composite to wet the substrate at the application temperature. If the volume of the glass phase is significantly greater than the available void volume, the base layer may not retain the desired degree of dimensional integrity during the application process. The available void volume is expected to depend on the prefiring size distribution associated with the refractory particles and the shape or shapes associated with the refractory particles. For example, uniform, highly acicular needles are expected to pack less efficiently than a broadly distributed population of spherical particles. Useful ranges of the ratio of glass phase volume to refractory phase volume within the base layer are from about 1.2:1 to about 3.0:1. The weight percentage of refractory particles in a label layer after firing is in the range of 4.7% to 33.3% for most label applications.

Particle size as used herein refers to the longest dimension of a particle, and can be measured by any convenient technique. For particles up to about 100

micrometers in size, a "Coulter Counter, Model TA3" particle size analyzer available from Coulter Corp., Hialeah, FL, can be used.

Glassy phases 20 and 24, or any glass phase to be used in labels according to the present invention, may be independently formed from a wide variety of glassy materials, or combination of glassy materials. Most typically, glassy phases 20 and 24 each independently comprise one or more metal oxides of Si, Al, Fe, K, Mg, Ti, P, Ca, Na, Mn, B, Ge, Zn, Sn, Ba, Bi, Cu, Li, combinations thereof, and the like, according to formulaic characterization in which glass composition is expressed on a theoretical oxide basis (e.g., in the case of Si as SiO_2). Incorporating various amounts of oxides of the Sn, Zn, and Li into the glassy phases is particularly useful for controlling the respective softening points. For example, using greater amounts of SnO, graphite, and/or Li_2O tends to lower the softening point (graphite also tends to lower the softening point, but is a refractory material), whereas using greater amounts of SnO tends to increase the softening point. Incorporating P_2O_5 generally tends to mechanically strengthen a glassy phase.

It is particularly preferred that glassy phases 20 and 24 be substantially free of any of the following heavy metal atoms: Pb, Cd, As, Hg, Co, and Cr(VI). By using oxides of SnO and Li_2O, the present invention advantageously achieves low softening points without relying upon oxides of the less desirable heavy metals. Also in the past, oxides of heavy metals such as Co and Cr were used for providing black color. Embodiments of the present invention avoid these heavy metals by using other black-colored particles as a substitute. Graphite, for example, not only tends to lower the softening point but is also an excellent dark colorant.

As concrete examples, glassy phase 20 preferably comprises, on a theoretical oxide basis, in the range from about 20 to about 40 percent by weight SnO, in the range from about 20 to about 35 percent by weight SnO, and in the range from about 35 to about 45 percent by weight P_2O_5, based on the total weight of glassy phase 20. Glassy phase 24 preferably comprises, on a theoretical oxide basis, in the range from about 20 to about 37 percent by weight SnO, in the range from about 24 to about 35 percent by weight SnO, and in the range from about 39

to about 44 percent by weight P_2O_5, based on the total weight of glassy phase 24. Either of these preferred glassy phases 20 and/or 24 may also include up to about 1 percent by weight of Li_2O.

As an option, base layer 14 and/or top layer 16 may further incorporate glass and/or refractory fibers in order, for example, to increase toughness. Glass and/or refractory fibers suitable for this purpose may have a wide range of particle sizes. Generally, fibers having an average diameter in the range 10 to 20 micrometers, preferably 13 to 16 micrometers, and an aspect ratio (i.e., length to diameter) of 3 to 20 would be suitable. A specific example of one suitable glass fiber having an average diameter of 13.3 micrometers and an aspect ratio of about 10 is available under the trade designation "739 EC1/32" from Owens Corning Co., of Anderson, SC.

Generally, a sufficient amount of fibers may be added to improve toughness. Typically, using 20 to 30 parts by weight of glass fibers per 100 parts by weight of glassy phase would be suitable. Typically, the glass fiber is a substitution for a corresponding amount of glass frit and refractory materials, the glass fiber usually having a softening point significantly higher than that of glass frit.

In preferred embodiments of the present invention, color contrast characteristics are provided by incorporating appropriately colored refractory particles into each of base layer 14 and top layer 16. However, a suitable degree of color contrast can be provided by other approaches. For example, one or both of layers 14 and 16 may incorporate particles that fluoresce in response to the light emitted from a bar code scanning device so that the optical discernibility between layers 14 and 16 is thereby enhanced. In such embodiments, the code pattern may or may not be optically discernible in the absence of the scanner's interrogation beam, but becomes optically discernible to the scanner when the particles fluoresce. This approach relies upon the appreciation that the label need only display the requisite color contrast at the time of scanning.

Representative examples of fluorescent particles responsive to the scanning light of a bar code scanner include particles that fluoresce in response to the light, radiation, or other stimulus emitted from the scanning device so that they

are optical discernible. Representative examples include UV excited fluorescent lamp phosphors or visible light responsive phosphorescent materials such as alkaline earth halophosphates (e.g., obtained from Sylvania under the trade designation "COOL WHITE CA HALOPHOSPHATE NO. 4459"), zinc orthosilicates, and sulfides such as ZnS and CaS. Rare earth doped yttria, or yttrium, gallium, and aluminum garnets and other oxides or oxysulfides are additional phosphor materials. Typically, these particles have a particle size in the range from 1 to 100 micrometers, often 2-20 micrometers. A sufficient amount of such particles is included in base layer 14 and/or top layer 16 so that the code pattern is optically discernible at the time that the code pattern is "read" by a suitable reading device. General, using 5 to 500 parts by weight of phosphor particles based on 100 parts by weight of the corresponding glassy phase would be suitable.

Label 10 according to the present invention may be provided with an overall thickness within a wide range depending upon factors such as the nature of substrate 12, the type of code pattern used on the label, the purpose of the label, and the like. Practically, the label should be thick enough to withstand the rigors of its intended use while still being pliably conformable to nonplanar substrates, but not so thick that the label unduly gets in the way when handling the substrate or is otherwise inconvenient. Further, extra thickness may offer little added benefit in terms of mechanical strength while adding unnecessary bulk, cost, or the like. Generally, it has been found that providing the label with an overall thickness in the general range from 100 micrometers to about 2 mm, preferably 400 to 800 micrometers, is suitable for many applications.

The embodiments of the present invention shown in FIGS. 1 and 3 are particularly suitable for forming labels in which a code pattern is formed in top layer 16 using laser ablating techniques. Accordingly, top layer 16 of such embodiments is desirably provided with a thickness such that laser ablating removes a sufficient amount of top layer 16 to expose the color contrast of the underlying layer, thereby allowing the resultant code pattern to be optically discernible. If top layer 16 is too thick, the laser may not penetrate far enough into top layer 16 so as to provide an effective code pattern. If top layer 16 is too thin,

the laser may penetrate not only through top layer 16, but may also too far into the underlying structure so as to affect the structural integrity of label 10. Substrate 12 could also be damaged as well.

Balancing these concerns, an appropriate thickness for top layer 16 can be determined empirically by measuring the depth to which the etching laser penetrates into label 10. The thickness of top layer 16 may then be selected to substantially correspond to that depth. Preferably, however, the thickness of top layer 16 is slightly less than the depth of laser penetration into label 10 so that the laser penetrates a little ways into the underlying ceramic body 13. This fully exposes contrasting portions of the underlying body 13 to maximize the optical discernibility of the code pattern. Generally, penetration into the underlying body 13 may be up to 10%, preferably up to 5%, more preferably up to about 1% of the total thickness of underlying body 13. Typically, providing top layer 16 with a thickness in the range from 5 micrometers to about 80 micrometers would be suitable in the practice of the present invention.

FIGS. 4 and 5 show alternative embodiments of the present invention which are identical to the embodiments of FIGS. 2B and 3, respectively, except for two differences. Firstly, top layers 16D and 16E of FIGS. 4 and 5, respectively, each comprises a refractory phase that has been replaced by a metal, an intermetallic material, and/or a metal alloy, that is preferably a noble metal such as gold, silver, platinum, and the like. As used hereinafter, the term "metal" with respect to top layer 16D and 16E will refer to a metal, an intermetallic material, and/or a metal alloy. In FIG. 4, top layer 16D may be formed upon composite ceramic base layer 14D using any suitable technique for depositing metal-containing coatings. Representative techniques for forming such a metal coating include sputtering, chemical vapor deposition, screen printing, electroless plating, chemical reduction, photo reduction, and the like. In FIG. 5, top layer 16E is selectively patterned to incorporate a code pattern. As is the case with the embodiment shown in FIGS. 2A and 2B above, the code pattern of metal top layer 16E in FIG. 5 may be formed by abrading, ablating, or otherwise etching top layer 16E. Alternatively, rather than using abrading, ablating, or etching techniques to pattern metal top layer 16E of Fig 5, the patterned form of top layer 16E shown in

FIG. 5 can be formed directly using a suitable printing technique such as an ink jet printing with a metal containing ink or a metal deposition through a mask (see, e.g., U.S. Pat. No. 5,407,474 referred to above).

As a second difference, because top layers 16D and 16E of FIGS. 4 and 5, respectively, each are formed from a metal, top layer 16D and 16E will generally tend to have a relatively dark color as a natural characteristic. Accordingly, base layer 14D and 14E desirably incorporates relatively light colored refractory particles 32D and 32E so that the code pattern formed in top layer 16D and 16E is optically discernible from base layer 14D and 14E. When top layers 16D and 16E of FIGS. 4 and 5, respectively, each are formed from a metal, it optionally may also be possible to discern the encoded information by detecting variations in electrical or thermal conductivity across the face of the patterned label.

In embodiments of the present invention incorporating base layer glassy phase 20 having a relatively low softening point (e.g., a softening point less than about 350°C), firing of such embodiments when green can result in organic constituents becoming trapped in glassy phase 20. Such entrapment can cause base layer 14 to be black in color. Accordingly, as one option for such embodiments, it is preferred to use such black base layers 14 in combination with a contrasting, (e.g., white), top layer 14. This ensures that label 10 will comprise two layers of contrasting color so that an optically discernible code pattern can be formed.

In some applications, however, the use of black colored top layer 16 is still desired, notwithstanding the black color of base layer 14. Unfortunately, such a black-on-black structure is not an optimum configuration for labeling purposes due to the lack of contrast between the two layers. The embodiment of the present invention shown in FIG. 6 provides a solution to this problem. There, label 10F is identical to label 10C of FIG. 3 with two exceptions. First, base layer 14F further incorporates organic constituents helping to make base layer 14F black in color. Secondly, body 13F of label 10F further incorporates relatively light colored intermediate layer 30 interposed between dark base layer 14F and dark top layer 16F. Intermediate layer 30 comprises a glassy phase 32 and a refractory phase in the form of relatively light refractory particles 34 interspersed in glassy phase 32. Glassy phase 32 and refractory particles 34 may be as defined above with respect to

FIGS. 1-3. However, it is preferred that glassy phase 32 have a softening point greater than that of glassy phase 20F and that glassy phase 24F have a softening point greater than that of glassy phase 32F. In other words, each layer of label 10F preferably incorporates a glassy phase having a higher softening point than any of the glassy phase(s) in the underlying layer(s). More preferably, the softening point of glassy phase 32 is 5°C to about 50°C, preferably 5°C to 20°C, greater than the softening point of glassy phase 20, and the softening point of glassy phase 24 is 5°C to about 50°C, preferably 5°C to 20°C, greater than the softening point of glassy phase 32F.

Labels according to the present invention may be made using a variety of approaches. For example, a base layer may be obtained from a slurry incorporating refractory particles 22, a glass frit corresponding to glassy phase 20, a polymeric binder, and a solvent. Optional ingredients include an organic plasticizer, dispersants to facilitate dispersion of the refractory and glass frit particles, and the like. If the particle sizes of the refractory particles and/or the glass frit are too large at the outset, particle sizes may be reduced to the desired size using any suitable size reducing technique in the art such as crushing, wet ball milling, dry ball milling, jet milling, and the like.

The solvent may be water or a nonaqueous liquid, or combinations thereof. The preferred solvent is a nonaqueous liquid such as dichloromethane, trichloroethane, methyl ethyl ketone, toluene, ethyl alcohol, combinations of these, and the like. Typically, the slurry includes from about 40 to about 70 percent by volume of the solvent, based upon the total volume of the slurry ingredients.

The polymeric binder and organic plasticizer, if any, may be any of those materials known in the art to aid in the formation of a green article. The term "green" with respect to a ceramic article means that the ceramic article includes organic constituents and is not heat-treated or is incompletely heat-treated. Useful organic plasticizers include glycerol, polyethylene glycol, dioctyl phthalate, combinations of these, and the like. The weight ratio of the plasticizer to total weight of glass frit and refractory particles is typically in the range from about 1:10 to about 1:25. Suitable polymeric binders include acrylic polymer, polyvinyl butyral, polyvinyl alcohol, and the like, and may be selected depending on the

solvent being used in accordance with conventional practices. The weight ratio of polymeric binder to total weight of glass frit and refractory particles is typically in the range from about 1:9 to about 1:15.

The slurry may further comprise dispersing aids known in the art. Representative examples of commercially available dispersants include a sodium salt of a carboxylate polyelectrolyte dispersant (available, for example, under the trade designation "TAMOL 731" from Rohm & Haas Co., Philadelphia, PA). Another useful additive is a defoaming agent (available, for example, under the trade designation "SURFYNOL 104E" from Air Products Co., Allentown, PA).

To form the slurry, the ingredients can be combined in a suitable mixing apparatus, such as a media mill, and mixed until the slurry has a viscosity appropriate for casting. To remove air which may be trapped in the slurry as a result of mixing, the slurry is preferably deaerated under a vacuum, for example, by use of an aspirator or vacuum pump, prior to forming the slurry into the shape of a base layer sheet. A suitable vacuum is about 1 torr. Deaerating may also occur, for example, by slow speed (e.g., 1 rpm) rolling on a roller.

After the slurry is formed and optionally deaerated, the slurry can be formed into the desired shape (e.g., a sheet or wafer shape) using conventional techniques such as tape casting, slip casting, screen printing, gravure coating, knife coating, and the like. The preferred method for shaping the slurry is tape casting. Tape casting is a conventional method that utilizes a doctor blade or knife blade to coat the slurry onto a release liner. To accomplish tape casting, the viscosity of the slurry is preferably in the range from about 2000 to 2900 cps, as determined under ambient conditions using a Brookfield viscometer fitted with a No. 3 spindle at 100 rpm. If the viscosity of the slurry is too low after milling, the viscosity can be increased by removal of a portion of the solvent prior to tape casting. Typically, solvent is removed from the slurry by evaporation during mixing.

Typically, a sheet is first cast onto a carrier support, then carefully dried to produce an uncracked, unwarped green article, which is subsequently cut into wafer-shaped pieces. Drying can be accomplished using any of several conventional liquid media removal techniques including heating. Preferably, the green label pieces are dried in air at room temperature or heated in air at a

temperature in the range from about 30°C to about 50°C. The thickness of a green tape after drying is typically in the range from about 0.1 mm to about 2 mm.

Additional layers may be formed on the green base layer by coating, laminating, or ink jet printing, or the like. For example, a slurry of ingredients as described above except having ingredients corresponding to those of the green, additional layer to be coated can be applied to a surface of the underlying, green pieces. A typical wet coating thickness is in the range from 3 micrometers to 20 micrometers. The coating slurry can be applied to the surface using techniques known in the art as listed above. The coated article is then preferably dried. The coating and drying process may be repeated to add more layers as desired. After the last layer has dried, the multilayer, green structure may be cut into desired shapes or pieces.

In an alternative approach for forming the multilayer green label, green sheets of each layer can be separately prepared using the procedures described above. The sheets, whether two or more, can then be stacked and laminated together using appropriate pressure and temperature. For example, a lamination pressure of 3000 psi (20.7 MPa) and lamination temperature of 65°C have been found to be suitable for some compositions. After lamination, the multilayer, green structure can be cut into the desired pieces. According to another approach, additional metal, ceramic, and/or glass coatings can be formed on the base layers using ink jet printing techniques. When using ink jet printing techniques, a patterned top layer can be directly formed without intervening abrading, ablation, or etching steps.

Firing of the green, dried, multilayer label pieces preferably occurs under conditions such that the resultant fired label includes substantially no organic constituents other than carbon. More preferably, firing occurs under conditions such that the resultant fired label is at least partially sintered, and more preferably under conditions such that the resultant fired label is substantially fully sintered. A substantially fully sintered article refers to a fired article having a density of at least 80%, preferably at least 85%, more preferably at least 90%, and even more preferably at least 95% of theoretical.

To accomplish firing, the green article may be heated from room temperature to the desired maximum firing temperature under conditions effective to burn out organic constituents without causing the article to suffer from undue thermally induced damage. The preferred heating rate is dependent upon a variety of factors including the atmosphere in which the article is heated, the amount and type of organic substituent present, and the desired color of the base material to be fired. Preferably, the heating rate is slow enough to avoid cracking, bloating, undue shrinkage, or distortion of the green article during firing. The heating rate should also occur at a rate slow enough to prevent carbon trapping in label embodiments including a white base layer. For embodiments including a black base layer, carbon trapping might add black color to the base layer, so a faster heating rate can be used so long as the label maintains dimensional and structural integrity during firing. Preferably, firing occurs in air. Firing may occur by heating the green article to the maximum firing temperature in one or more heating stages, as desired.

Preferably, the maximum temperature to which the green article is heated during firing is sufficiently greater than the softening point(s) of the glassy phase(s) incorporated into the green article to facilitate sintering without loss of dimensional and/or structural integrity of the article. If the difference between the maximum firing temperature and the softening point of a particular glassy phase is too small, then that glassy phase might be too porous after firing, and therefore too weak mechanically. On the other hand, if the difference between the maximum firing temperature and the softening point of a particular glassy phase is too great, then the viscosity of that glassy phase might be too low during firing and/or during attachment to a substrate. The latter can cause such a glassy phase to flow out from the label too much, leading to excessive stickiness and/or a loss of structural and/or dimensional integrity.

Accordingly, in preferred embodiments, the maximum firing temperature is selected to be in the range from about 25 °C to about 300 °C, preferably 50 °C to about 150 °C, greater than the softening point(s) of the glassy phases incorporated into a label according to the present invention. Given a desired application temperature, the maximum firing temperature is also desirably less than the desired

application temperature. For example, using a maximum firing temperature that is 50°C to 250°C below the application temperature would be suitable. To illustrate examples of such relationships, the following table shows suitable ranges of firing temperatures for corresponding softening point and application temperatures:

Application Temperature	Firing Temperature Range	Softening Point Range
550°C	300°C to 500°C	250°C to 450°C
750°C	500°C to 700°C	450°C to 650°C
800°C	550°C to 750°C	500°C to 700°C

After firing, a desired code pattern can then be formed in the top layer (unless the top layer is already patterned (e.g., because ink jet printing or a similar technique was used), as desired, either before and/or after the label is attached to its substrate. The code pattern preferably comprises alphanumeric information, bar code information, pictorial information, Braille, other identifiable characters, combinations of these, or the like. The code pattern may be human readable, machine readable (e.g., readable by a bar code scanner), combinations of these, or the like. In embodiments in which the code pattern comprises a bar code, representative examples of the many varieties of suitable bar codes include 2 of 7 Code, 3 of 9 Code, ACI, AGES, Ames, Anker, AS-6, AS-10, Bar/Half Bar, Bi-Level Code, Binary Code, Binary Coded Decimal, Bulls Eye Code, Codabar, Code 11, Code 39, Code 93, Code 128, Code B, Color Bar Code, Delta Distance A, Delta Distance B, Delta Distance C, Digital Code, Distribution Symbol, Double Track Symbol Code, European Article Numbering (EAN), F2F, Frequency Code, Fujitsu, IER, International Article Numbering (IAN), Interleaved 2 of 5, Matrix Two of Five, Modified BCD, MSI Bar Code, Nixdorf, Norand, Octal Coded Decimal, Periodic Binary, Plessary Code, Presence/Absence Code, PWM, RTC, Scope Code, Siemens Code, Sunburst Bar Code, Telepan, Ten Segment Decimal Code, Toshiba, Two of Five, Two of Five Matrix, Universal Product Code (UPC), USD-1, USD-2, USD-3, USD-4, USD-5, USD-6, USD-7, USD-8, Wagon Wheel Code, World Product Code (WPC), and Zellweger Code. Of these, bar codes that

are widely used include Code 39, UPC, EAN, 2 of 5, Interleaved 2 of 5, Codabar, and Plessey Code.

The resultant code pattern provides a convenient method of identifying the substrate to which the label bearing the code pattern is attached. For example, if the code pattern includes a bar code, a bar code scanner can be used to rapidly and accurately read the code pattern in order to identify the corresponding substrate. As is well known in the art, a typical bar code includes a number of printed bars and intervening spaces. The widths of the bars and spaces, as well as the number of each, correspond to different characters. The meaning to be attributed to such features is given by the specification for a particular bar code. Bar codes can encode letters, numbers, and other graphic symbols. Most typically, bar codes encode primarily numeric information.

In a typical bar code scanning operation, either a stationery or a hand-held bar code scanner emits an interrogation beam of electromagnetic radiation (e.g., light) which is swept across the bar code. Laser light is commonly used as the interrogation beam, although other forms of light may be used. A suitable light source may comprise IR light, visible light, UV light, or the like. To ensure that the bar code characters can be read with good resolution, the width of the interrogation beam is preferably no greater than about 1.4 times the minimum width of the thinnest bar code character. When the interrogation beam hits the bar code, the beam is reflected back towards the scanner. The scanner includes componentry for detecting the light intensity of the reflected beam. Portions of the interrogation beam reflected from dark characters or portions of the bar code will have less intensity than portions of the interrogation beam reflected from light characters or portions of the bar code. Thus the light reflected from the bar code will vary in intensity in a manner corresponding to the light and dark characters or portions of the bar code. A signal processing system included in the scanner, or operationally coupled to the scanner, then translates such variation in the reflected light intensity in order to decode the bar code. For additional information regarding bar codes see, for example, Craig K. Harmon and Russ Adams, Reading Between the Lines, An Introduction to Bar Code Technology, Chapter 3 (Helmers Publishing, Inc., 1984).

In some instances, it may be desirable to update the code pattern formed in labels according to the present invention. For example, during the course of production of cathode ray tube glass components, different components may be subjected to a different sequences of processing conditions depending upon the desired end uses of those components. In order to keep track of what sequence of conditions was used for each component, it may be desirable to update the label attached to the component with one or more additional code patterns corresponding to the one or more different processing conditions applied to the substrate. Thereafter, the code patterns on a label can be read and decoded to quickly and accurately determine the production history of the corresponding component.

The present invention will now be further described with reference to the following examples.

Example 1

A 2600 cm^3 porcelain ball mill jar with a diameter of about 18.0 cm was charged with about 1400 grams of alumina grinding media (1.5 cm diameter; commercially available from U.S. Stoneware Co. of Mahwah, NJ), about 600 grams of trichloroethane, about 50 grams of methyl ethyl ketone (MEK), 40 grams of dioctyl phthalate (commercially available under the trade designation "DOP" from Aldrich Chemical Co. of Milwaukee, WI), 36 grams of isopropanol, 50 grams of methylene chloride, 97.5 grams of a first glass frit (available under the trade designation "EG2712" from Ferro Corp., Cleveland, OH; softening point of 650°C; coefficient of thermal expansion of 8.0 x 10^{-6}/°C), 97.5 grams of a second glass frit (available under the trade designation "EG3012" from Ferro Corp.; softening point of 471°C; coefficient of thermal expansion of 11.8 x 10^{-6}/°C), and 105 grams of high purity mullite powder (Baikawski International Corp., Charlotte, NC). Both frits were free of the undesirable heavy metals.

The ingredients were ball milled at a speed of about 100 rpm for about four hours. About 60 grams of an acrylic copolymer latex emulsion (available under the trade designation "RHOPLEX B-60A" from Rohm and Haas, Philadelphia, PA), was added to the ball mill. The ingredients were then ball milled for an additional three hours.

About 150 milliliters of the resulting ball-milled slurry was transferred to a three neck flask (250 ml capacity) and placed under vacuum at 1 torr using a vacuum pump to deaerate while the slurry was stirred. The slurry thickened during stirring as liquid media evaporated. When the viscosity of the slurry increased to about 2900 cps measured at about 25°C using a Brookfield viscometer fitted with a No. 3 spindle operated at 100 rpm, the deaerated slurry was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting machine. The casting was allowed to dry at room temperature for about 10 hours.

The resultant dried, green casting was then stripped from the polyester film to provide a green sheet with a thickness of about 300 micrometers to 400 micrometers. The term "green" means that the structure included organic constituents. In terms of the actual, visually observable color, the mullite constituent provided the green sheet with a white color. The green sheet was cut into a number of 3.2 cm by 2.4 cm base layer pieces and a number of 2.0 cm by 8.0 cm base layer pieces. Black colored enamel paste (an oil base enamel available under the trade designation "VERSA COLOR" from American Art Clay Co., Inc., Indianapolis, IN) was then knife coated onto one side of each dried green base layer piece at a wet coating thickness of about 50 - 100 micrometers. On some samples, the black colored enamel paste was painted onto one side of each dried green base layer piece with a wet coating thickness of about 50-100 micrometers. The coated green samples were dried in an oven at about 70°C for about 3 hours.

The resulting green labels were then fired in a conventional resistive furnace in air according to the following firing schedule:

(1) heated from room temperature to 350°C at 2°C/minute;

(2) held at 350°C for one hour;

(3) heated from 350°C to 675°C at 2°C/minute; and

(4) held at 675°C for 24 minutes.

The linear shrinkage of the fired composite ceramic label articles was about 12 percent. The camber of the fired composite ceramic label articles was less than about 0.05 mm. The densities of the fired composite ceramic label articles were

about 1.5-1.7 g/cm^3. The colors of the base layer and top coating layer of the fired composite ceramic labels were white and black, respectively.

To demonstrate application of the coated ceramic labels to hot cathode ray tube glasses at an application temperature of 750°C, flat cathode ray tube glass pieces (from Philips Corp. of Aachen, Germany) were heated to 750°C in a conventional resistive furnace. The cathode ray tube glass pieces were each about 5 cm x 10 cm x 0.6 cm and had a coefficient of thermal expansion of about 9.8 x 10^{-6}/°C. The composition of the cathode ray tube glass pieces were as follows (in weight percent): SiO_2 - 53.5; Al_2O_3 - 5.2; Na_2O - 6.7; K_2O - 7.8; MgO+CaO - 1.75; PbO - 23.5; SrO - 0.5; BaO - 0.6; Sb_2O_3 - 0.3; Fe_2O_3 - <0.35; and the remainder at <0.2. After the cathode ray tube glasses reached 750°C, samples of the fired ceramic label articles were directly placed on the hot cathode ray tube glasses. The temperature was held at 750°C for about 10 minutes before the furnace was turned off and the samples were allowed to cool down to room temperature over about one hour. The samples were examined to evaluate adhesion of the fired ceramic labels to the cathode ray tube glasses. All of the labels were observed to be tightly bonded to the surface of the cathode ray tube glasses.

Example 2

A 1000 cm^3 porcelain ball mill jar with a diameter of about 13.0 cm was charged with about 500 grams of alumina grinding media (1.5 cm diameter), about 300 grams of trichloroethane, about 25 grams of MEK, 20 grams of dioctyl phthalate, 18 grams of isopropanol, 25 grams of methylene chloride, 48.75 grams of a first glass frit ("EG2712"), 48.75 grams of a second glass frit ("EG3012"), and 52.5 grams of monoclinic zirconia powder (commercially available as "HPZ 1000" from TAM Ceramics Co. Niagara Falls, NY). The ingredients were ball milled at a speed of about 100 rpm for about four hours. About 30 grams of acrylic copolymer latex emulsion ("RHOPLEX B-60A") was added to the ball mill. The ingredients were then ball milled for an additional three hours.

About 80 ml of the resulting ball milled slurry was transferred to a three neck flask (100 milliliter capacity) and placed under vacuum at 1 torr using a

vacuum pump to deaerate while the slurry was stirred. The slurry thickened during stirring as liquid media evaporated. When the viscosity of the slurry increased to about 2900 cps as measured in accordance with the viscosity measuring procedure of Example 1, the deaerated slurry was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting machine. The cast slurry was dried at room temperature in air for about five hours. The cast tape was then stripped from the polyester film to provide a green sheet having a thickness of 350 micrometers. The green sheet was cut into 3.2 cm by 2.4 cm base layer pieces and 2.0 cm by 8.0 cm base layer pieces. Black colored enamel paste ("VERSA COLOR") was then brush coated onto one side of the dried green base layer pieces to give a wet coating thickness of about 50 micrometers. The coated green samples were dried in an electric oven at about 70°C for about three hours.

The resulting green labels were then fired in a conventional resistive furnace in air according to following firing schedule:

(1) heated from room temperature to 270°C at 1°C/minute;

(2) held at 270°C for one hour;

(3) heated from 270°C to 650°C at 1°C/minute; and

(4) held at 650°C for 24 minutes.

The linear shrinkage of the fired composite ceramic label articles was about 12 percent. The camber of the fired composite ceramic label articles was less than 0.05 mm. The densities of the fired composite ceramic label articles were about 1.7-1.8 g/cm^3. The colors of the base layer and top coating layer of the fired labels were white and black, respectively.

To demonstrate application of one of the coated ceramic label samples to hot cathode ray tube glasses at an application temperature of 800°C, a flat cathode ray tube glass piece (see Example 1) was heated to 800°C in a conventional resistive furnace. After the cathode ray tube glass reached 800°C, the fired ceramic label article was directly placed onto the hot cathode ray tube glass and held at 800°C for about five minutes before the furnace was turned off and cooling was allowed to occur down to room temperature over about 45 minutes. The sample was observed to be tightly bonded to the surface of the cathode ray tube glass.

Example 3

Coated green labels were prepared using the procedure of Example 2, except that titania powder (commercially available under the trade designation "TiONA TiO_2" from SCM Chemicals Co., Baltimore, MD) was used in place of the zirconia. The resulting green label articles were then fired in a conventional resistive furnace in air according to following firing schedule:

(1) heated from room temperature to 250°C at 1°C/minute;

(2) held at 250°C for 2 hours;

(3) heated from 250°C to 650°C at 1°C/minute; and

(4) held at 650°C for one hour.

The linear shrinkage of the fired composite ceramic label articles was about 12 percent. The camber of the fired composite ceramic label articles was less than about 0.05 mm. The densities of the fired composite ceramic label articles were about 1.6-1.8 g/cm^3. Colors of the base layers and top coating layers were white and black, respectively.

To demonstrate application of one of the coated ceramic labels to hot cathode ray tube glasses at an application temperature of 750°C, a curved cathode ray tube glass piece (see Example 1) was heated to 750°C in a conventional resistive furnace. The diameter of the cathode ray tube glass piece was about 7.5 cm and its thickness was about 0.6 cm. After the cathode ray tube glass reached 750°C, a fired ceramic label article was directly placed onto the hot cathode ray tube glass before the furnace was turned off and cooling was allowed to occur down to room temperature over 30 minutes. The label easily conformed to the curved surface of the cathode ray tube glass. The ceramic label was observed to be tightly bonded to the surface of the cathode ray tube glass.

Example 4

This example describes the preparation of fired two layer ceramic labels which include a top, white coating layer and a black base layer and that can be fused to hot cathode ray tube glasses at application temperatures in the range from about 470°C to about 570°C.

A 1000 cm^3 porcelain ball mill jar with a diameter of about 13.0 cm was charged with about 500 grams of alumina grinding media, about 300 grams of trichloroethane, about 25 grams of MEK, 20 grams of dioctyl phthalate ("DOP"), 18 grams of isopropanol, 25 grams of methylene chloride, and 97.5 grams of a glass frit (obtained from Ferro Corp. under the trade designation "DPM003" and having the composition (in weight percent) as follows: SnO-54.3, ZnO-9.5, and P_2O_5-36.3); softening point of 293°C; coefficient of thermal expansion of 10.8 x 10^{-6}/°C). This glass frit included no Pb, Hg, As, Co, Cd, or Cr(VI). The ingredients were ball milled at a speed of about 100 rpm for about four hours. About 30 grams of copolymer latex emulsion ("RHOPLEX B-60A") were then added to the ball mill, and the ingredients were ball milled for an additional three hours. About 80 ml of the resulting slurry mixture was transferred to a flask. About 40 grams of silicon carbide powder (commercially available as "UF-05" from H.C. Starck Inc., Newton, MA), and 12.5 grams of black CoCrFeNi pigment (available as "F-3794" from Ferro Corp.), were added to the resulting ball-milled slurry and stirred at about 300 rpm using a conventional air propelled mixer having a 3.2 centimeter blade. About 80 ml of the resulting slurry mixture was transferred to a three neck flask (100 milliliter capacity) and placed under vacuum at 1 torr using vacuum pump to deaerate while the slurry was stirred. The slurry thickened during stirring as liquid media evaporated. When the viscosity of the slurry increased to about 2900 cps when measured as described in Example 1, the deaerated slurry was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting machine.

The cast slurry was dried at room temperature in air for about five hours. The cast tape was then stripped from the polyester film to provide a green sheet having a thickness of 350 micrometers. The green tape was cut into 2.0 cm by 8.0 cm base layer pieces. White color enamel paste was then brush coated onto one side of the dried green base layer pieces at a wet coating thickness of about 50 micrometers. The paste was a 70/30 wt. % mixture of white enamel powder and squeegee oil (available under the trade designation "A4" from American Art Clay Co. Inc.). The coated, green base layer pieces were dried in an electric oven at about 70°C for about three hours.

The resulting green label articles were then fired in a conventional resistive furnace in air according to following firing schedule:

(1) heated from room temperature to 400°C at 5°C/minute; and

(2) held at 400°C for 24 minutes before cooling down to room temperature over two hours.

The linear shrinkage of the fired composite ceramic label articles was about 12 percent. The camber of the fired two-layer ceramic labels was less than 0.05 mm. The density of the fired two-layer ceramic labels was about 1.6 g/cm^3. The colors of the base layers and top coating layers of the fired labels were black and white, respectively.

To demonstrate application of one of these coated ceramic labels to a hot cathode ray tube glass at an application temperature of about 530°C, a flat cathode ray tube glass piece (see Example 1) of about 5 cm x 10 cm x 0.6 cm was heated to about 530°C. A fired ceramic label was directly placed on the hot cathode ray tube glass, and the temperature was held at 530°C for about five minutes before the furnace was turned off and cooling was allowed to occur down to room temperature over about 30 minutes. The ceramic label was observed to be tightly bonded to the surface of the cathode ray tube glass.

Example 5

A 450 cm^3 glass jar with a diameter of about 7.0 cm was charged with about 100 grams of glass balls (50% of glass balls with a diameter of 16 millimeters and 50% of glass balls with a diameter of 12.5 millimeters; available from Jencors company, Bridgeville, PA), 50 grams of a first glass frit ("EG3012"), 50 grams of a second glass frit ("EG2712"), 0.5 gram of a dispersant (commercially available under the trade designation "TAMOL 731" from Rohm and Haas Co.), 0.45 gram of a defoaming agent (available under the trade designation "SURFYNOL 104 E" from Air Products Co., Allentown, PA), 12.8 grams of acrylic copolymer latex emulsion binder ("RHOPLEX B-60A"), and 30 grams of deionized water. The ingredients were ball milled at a speed of 100 rpm for about five hours. Some of the resulting slurry was transferred to a 100 ml three neck flask which was evacuated to 1 torr using a vacuum pump to deaerate. The

deaerated slurry mixture was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting technique. The cast tape was allowed to dry at room temperature for about 10 hours. The dried tape was then stripped from the polyester film to provide a green sheet with a thickness of 150 micrometers. The green sheet was cut into 4.0" x 4.0" (about 10 cm x 10 cm) base layer pieces. In order to produce a black colored top layer with metal, the dried green base layer pieces were screen printed with a palladium/silver metal paste (commercially available under the trade designation "PD/AG 6120" from E.I. Dupont de Nemours and Company of Wilmington, DE). The screen printed metal paste was then dried in an oven at about 80°C for about 30 minutes.

The resulting two layer labels were then cut into 1.0" (2.5 cm) x 3.0" (7.5 cm) pieces and fired in a conventional resistive furnace in air according to the following schedule:

(1) heating from room temperature to 350°C at 2°C/minute;

(2) holding at 350°C for one hour;

(3) heating from 350°C to 675°C at 2 C/minute; and

(4) holding at 675°C for 24 minutes.

The colors of the base layers and top coating layers of the fired labels were white and black, respectively.

To demonstrate application of the two layer ceramic labels to hot cathode ray tube glass at an application temperature of 750°C, a flat cathode ray tube glass piece with a size of about 5 cm x 10 cm x 0.6 cm (see Example 1) was heated to 750°C in a conventional resistive furnace. After the cathode ray tube glass reached 750°C, a fired, two layer ceramic label was directly placed onto the hot cathode ray tube glass and held at 750°C for about 10 minutes before the furnace was turned off and the sample cooled down to room temperature over about one hour. The label was observed to be firmly bonded to the surface of the cathode ray tube glass.

Example 6

This example describes the fabrication of a two layer green label using lamination techniques.

First, a green base layer was produced by tape casting a slurry mixture which was ball milled through following procedure: A 450 cm^3 glass jar with a diameter of about 7.0 cm was charged with about 100 grams of glass balls (50% of glass balls with diameter of 16 millimeters and 50% of glass balls with diameter of 12.5 millimeters), 65 grams of a glass frit ("DPM003"), 2 grams of 100% pure corn oil (available under the trade designation "MAZOLA" from Best Foods, CPC International Inc., Englewood Cliffs, NJ), 167 grams of toluene, 7.5 grams of a polyethylene glycol (available under the trade designation "CARBOWAX METHOXY POLYETHYLENE GLYCOL 2000" from Union Carbide Co., Danbury, CT), and 12.4 grams of dioctyl phthalate ("DOP"). The ingredients were ball milled at a speed of about 100 rpm for about five hours. About 10.7 grams of polyvinyl butyral (Aldrich Chemical Co.), 28 grams of silicon carbide particles (Grade 1200, mean diameter of 4.9 micrometers, Superior Graphite Co., Chicago, IL), and 14 grams of graphite powder (Sherritt Gordon Mines LTD, Alberta, Canada) were added to the ball mill. The ingredients were then ball milled for an additional 2 hours. The resulting ball-milled slurry was transferred to an open beaker and stirred at about 300 rpm using a conventional air propelled mixer having a 3.2 centimeter blade. The slurry mixture thickened during stirring as liquid media evaporated. When the viscosity of the slurry mixture increased to about 2900 cps when measured as described in Example 1, the mixture was placed in a 100 ml three neck flask which was evacuated to 1 torr using a vacuum pump to deaerate. The deaerated slurry mixture was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting technique. The cast tape was allowed to dry at room temperature for about 8 hours. The dried tape was then stripped from the polyester film to provide a green sheet with a thickness of 500 micrometers. The green sheet was cut into 4.0" (10 cm) x 4.0" (10 cm) base layer pieces.

A top layer green tape was produced by tape casting a slurry mixture which was ball milled through following procedure: A 200 cm^3 glass jar was charged with about 50 grams of glass balls (16 millimeter diameter), 30 grams of a glass frit (obtained from Ferro Corp. under the designation "DPM004" and having the composition (in weight percent) as follows: SnO-36.4, ZnO-24.1, and P_2O_5-39.6);

softening point of 350°C, a coefficient of thermal expansion of 8.8 x 10^{-6}/°C; notwithstanding the low softening point, this glass frit included no Pb, Hg, As, Co, Cd, or Cr(VI)), 20 grams of titania powder ("TiONA TiO_2"), 6.4 grams of acrylic copolymer latex emulsion ("RHOPLEX B-60A"), 0.25 gram of dispersant ("TAMOL731"), 0.23 gram of defoaming agent ("SURFYNOL 104 E"), and 20.0 grams of deionized water. The ingredients were ball milled at a speed of 100 rpm for about three hours. The resulting slurry was transferred to a 100 ml three neck flask which was evacuated to 1 torr using a vacuum pump to deaerate. The deaerated slurry mixture was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting technique. The cast tape was allowed to dry at room temperature for about 8 hours. The dried tape was then stripped from the polyester film to provide a green sheet with a thickness of 8 micrometers. The green sheet was cut into 4.0" (10 cm) x 4.0" (10 cm) top layer pieces.

Two layer laminates were formed by laminating a base layer piece and a top layer piece together at a temperature of 65°C for 30 seconds at a pressure of 3000 psi (20.7 MPa). The two layer laminate was then cut into 1.0" (2.5 cm) x 3.0" (7.5 cm) label pieces.

The resulting green labels were then fired in a conventional resistive furnace in air according to the following schedule:

(1) heating from room temperature to 250°C at 2°C/min.;

(2) holding at 250°C for one hour;

(3) heating from 250°C to 400° at 2°C/min; and

(4) holding at 400°C for 0.4 hour.

The colors of base and top layers of the fired ceramic labels produced were black and white, respectively.

To demonstrate application of the two layer ceramic labels to hot cathode ray tube glass at application temperature of 530°C, a flat cathode ray tube glass piece with size of about 5.0 cm x 10.0 cm x 0.6 cm (see Example 1) was heated to 530°C in a conventional resistive furnace. After the cathode ray tube glass reached 530°C, a fired ceramic label was directly placed on the hot cathode ray tube glass.

The temperature was held at 530°C for about one minute before the furnace was turned off, and the sample was allowed to cool down to room temperature over about one hour. The label was observed to be firmly bonded to the surface of the cathode ray tube glass.

Example 7

A 450 ml glass jar with a diameter of about 7.0 cm was charged with about 100 grams of glass balls (50% of glass balls with a diameter of 16 millimeters and 50% of glass balls with a diameter of 12.5 millimeters), 65 grams of a first glass frit ("DPM003"), 15 grams of a second glass frit ("DPM004"), 20 grams of silicon carbide particles (Grade 1200, mean diameter of 4.9 micrometers, Superior Graphite Co., Chicago, IL), 15 grams of CoCrFeNi black pigment ("F-3794"), 0.5 gram of dispersant ("TAMOL 731"), 0.45 gram of defoaming agent ("SURFYNOL 104 E"), 12.8 grams of acrylic copolymer latex emulsion binder ("RHOPLEX B-60A"), and 30 grams of deionized water. The ingredients were ball milled at a speed of 100 rpm for about three hours. About 100 ml of the resulting slurry was transferred to a 100 ml three neck flask which was then placed under vacuum at 1 torr using a vacuum pump to deaerate. The deaerated slurry mixture was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting technique. The cast tape was allowed to dry at room temperature for about 10 hours. The dried tape was then stripped from the polyester film to provide a green sheet with a thickness of about 200 micrometers. The green sheet was cut into 1.0" (2.5 cm) x 4.0" (10 cm) base layer pieces.

For the production of a white colored top layer, a mixture of 4.3 grams of squeegee oil (available under the trade designation "A-4" from Thompson Enamel Co., Bellevue, KY), 7 grams of the glass frit used in the top layer of Example 6, and 3 grams of titania powder ("TiONA TiO_2") was brushed onto the base layer pieces at a coating thickness of about 20 micrometers.

The coated labels were then fired in a conventional resistive furnace in air according to the following schedule:

(1) heating from room temperature to 450°C at 5°C/minute; and

(2) holding at 450°C for 24 minutes.

The colors of the top and base layers of the fired samples were white and black, respectively.

To demonstrate application of the ceramic labels to hot cathode ray tube glass at an application temperature of 530°C, a curved cathode ray tube glass piece with a size of about 5 cm x 10 cm x 0.6 cm (see Example 1) was heated to 530°C in a conventional resistive furnace. After the cathode ray tube glass reached 530°C, a fired label was directly placed onto the hot cathode ray tube glass and held at 530°C for about 30 seconds before the furnace was turned off and the sample cooled down to room temperature over about one hour. The label was observed to be firmly adhered to the surface of the cathode ray tube glass.

Example 8

This example describes the fabrication of a three layer ceramic label using lamination and coating techniques.

An intermediate layer green tape was produced by tape casting a slurry mixture which was ball milled through following procedure: A 450 ml glass jar with a diameter of about 7.0 cm was charged with about 100 grams of glass balls (50% of glass balls with a diameter of 16 millimeters and 50% of glass balls with a diameter of 12.5 millimeters), 65 grams of a glass frit ("DPM004"), 35 grams of titania powder ("TiONA TiO_2"), 0.5 gram of dispersant ("TAMOL 731"), 0.45 gram of defoaming agent ("SURFYNOL 104 E"), 12.8 grams of copolymer latex emulsion ("RHOPLEX B-60A"), and 30 grams of deionized water. The ingredients were ball milled at a speed of 100 rpm for about five hours. About 100 ml of the resulting slurry was transferred to a 100 ml three neck flask which was then placed under vacuum at 1 torr using a vacuum pump to deaerate. The deaerated slurry mixture was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting technique. The cast tape was allowed to dry at room temperature for about 10 hours. The dried tape was then stripped from the polyester film to provide a green sheet with a thickness of 150 micrometers. The green tape thus produced was cut into 4.0" (10 cm) x 4.0" (10 cm) pieces. One of these green pieces was stacked on a black color, green base layer tape (thickness of 200 micrometers) produced in accordance with Example 7.

The two green pieces were laminated at a temperature of 65°C for 30 minutes at a pressure of 3000 psi (20.7 MPa). The two layer laminate was then cut into 1.0" (2.5 cm) x 4.0" (10 cm) pieces. For the production of a black colored top layer, a mixture of 4.3 grams of squeegee oil ("A-4"), 7 grams of a glass frit ("DPM004"), and 3 grams of black pigment powder ("F-3794" CoCrFeNi, Ferro Corp., Cleveland, OH) was brush coated onto the white layer of the green laminate at a thickness of about 20 micrometers. The three-layer coated label was then fired in a conventional electrically resistive furnace in air according to following schedule:

(1) heating from room temperature to 450°C at 5°C/minute; and

(2) holding at 450°C for 24 minutes.

The colors of the top, intermediate, and base layers were black, white, and black, respectively.

To demonstrate application of the ceramic label to hot cathode ray tube glass at an application temperature of 530°C, a curved cathode ray tube glass piece with a size of about 5 cm x 10 cm x 0.6 cm (see Example 1) was heated to 530°C in a conventional resistive furnace. After the cathode ray tube glass reached 530°C, the fired ceramic label was directly placed onto the hot cathode ray tube glass. The temperature was held at 530°C for about 30 seconds before the furnace was turned off and the sample was allowed to cool down to room temperature over about one hour. The label was observed to be firmly adhered to the surface of the cathode ray tube glass.

Example 9

To prepare a base layer tape, a 450 ml glass jar with a diameter of about 7.0 cm was charged with about 100 grams of glass balls (50% of glass balls with a diameter of 16 millimeters and 50% of glass balls with a diameter of 12.5 millimeters), 21.7 grams of a first glass frit ("DPM004"), 65 grams of a second glass frit ("DPM003"), 17.5 grams of graphite powder (Sherritt Gordon Mines LTD), 0.5 gram of dispersant ("TAMOL 731"), 0.45 gram of defoaming agent ("SURFYNOL 104 E"), 12.8 grams of acrylic copolymer latex emulsion binder ("RHOPLEX B-60A"), and 46 grams of deionized water. The ingredients were ball milled at a speed of 100 rpm for about three hours. About 15 grams of milled

glass fiber (available under the trade designation "739 EC 1/32" from Owens Corning Co., having an average diameter of 13.3 micrometers and an aspect ratio of 10) was added to the resulting ball-milled slurry. The ingredients were stirred at about 300 rpm using a conventional air propelled mixer having a 3.2 cm blade. About 100 ml of slurry mixture was then transferred to 100 ml three neck flask which was placed under vacuum of about 1 torr using a vacuum pump to deaerate. The deaerated slurry mixture was then uniformly cast onto a silicone-coated polyester film using a conventional doctor-blade tape casting technique. The cast tape was allowed to dry at room temperature for about 10 hours. The dried tape was then stripped from the polyester film to provide a green sheet with a thickness of about 300 micrometers. The green sheet was cut into 1.0" (2.5 cm) x 4.0" (10 cm) base layer pieces.

For the production of a white colored top layer, a mixture of 4.3 grams of squeegee oil ("A-4"), 6 grams of a glass frit ("DPM004"), and 4 grams of titania powder (TiONA TiO_2) was brush coated onto the base layer green pieces at a thickness of about 20 micrometers. The coated green label was then fired in a conventional resistive furnace in air according to the following schedule:

(1) heating from room temperature to 450°C at 5° /minute; and

(2) holding at 450°C for 24 minutes.

The colors of the top and base layers of the resultant fired ceramic label were white and black, respectively.

To demonstrate application of the two contrasting layer ceramic label to hot cathode ray tube glass at an application temperature of 530°C, a flat cathode ray tube glass piece with a size of about 5 cm x 10 cm x 0.6 cm (see Example 1) was heated to 530°C in a conventional resistive furnace. After the cathode ray tube glass reached 530°C, the fired label was directly placed onto the hot cathode ray tube glass and held at 530°C for about 10 seconds before the furnace was turned off. The sample was allowed to cool down to room temperature over about one hour. The label was observed to be firmly adhered to the surface of the cathode ray tube glass.

Example 10

A one gallon (3.8 liter) glass jar with a diameter of about 14.0 cm was charged with about 1000 grams of glass balls (50% of glass balls with a diameter of 16 millimeters and 50% of glass balls with a diameter of 12.5 millimeters), 800 grams of a glass frit ("DPM003"). 175 grams of graphite powder (Superior Graphite Co.), 9.4 grams of phosphate ester (available under the trade designation "EMPHOS PS21A" from Witco Corp., New York, NY), and 530 grams of a mixture of 66% by volume MEK with 34% by volume ethanol. The ingredients were ball milled at a speed of 100 rpm for about an hour. About 105 grams of polyvinyl butyral (Aldrich Chemical Co.), 70 grams of polyethylene glycol ("CARBOWAX METHOXY POLYETHYLENE GLYCOL 2000"), and 100 grams of dioctyl phthalate ("DOP") were added to the ball mill. The ingredients were then ball milled for an additional two hours.

The resulting ball-milled slurry was transferred to open beaker and about 175 grams of milled "739 EC 1/32" glass fiber having an average diameter of 13.3 micrometers and aspect ratio of 10 was added to the ball-milled slurry and stirred at about 300 rpm using a conventional air propelled mixer having a 3.2 cm blade. This slurry mixture was then transferred to a 1 gallon (3.8 liter) plastic bottle (available under the trade designation "NALGENE" from Nalgene of Chicago, IL) and slowly rolled at a speed of 1 rpm to get rid of air bubbles before casting onto a silicone-coated polyester film using a doctor blade tape casting machine (manufactured by AEM Co., San Diego, CA, under the trade designation "AEM 2104").

The dried green tapes were then stripped from the polyester film to provide green sheets with a thickness of about 175 micrometers. The green sheets were cut into 4.0" (10 cm) x 5.0" (12.5 cm) pieces. For the production of a white colored top layer, a half gallon (1.9 liter) glass jar with a diameter of about 12.0 cm was charged with about 500 grams of glass balls with a diameter of 16 millimeters, 350 grams of a glass frit ("DPM003"), 150 grams of titania powder ("TiONA TiO_2"), 4.7 grams of phosphate ester ("EMPHOS PS21A"), and 265 grams of solvent mixture of 66% by volume MEK with 34% by volume ethanol. The ingredients were ball milled at a speed of 100 rpm for about an hour. About 52.5 grams of

polyvinyl butyral (Aldrich Chemical Co.), 35 grams of polyethylene glycol ("CARBOWAX METHOXY POLYETHLENE GLYCOL 2000"), and 50 grams of dioctyl phthalate ("DOP") were added to the ball mill. The ingredients were then ball milled for an additional two hours. The resulting ball-milled slurry was transferred to a half gallon (1.9 liter) plastic bottle ("NALGENE")and slowly rolled at a speed of 1 rpm for deaeration before casting onto a silicone coated polyester film using a tape casting machine ("AEM 2104"). The dried green tapes were then stripped from the polyester film to provide green sheets with a thickness of about 80 micrometers. The green sheets were cut into 4.0" (10 cm) x 5.0" (12.5 cm) pieces. One of these white-colored pieces was placed onto four black colored pieces produced above to form a label having 5 layers. The stack was laminated at a temperature of 65°C for 5 seconds at a pressure of 220 psi (1.5 MPa). The laminates were then cut into 12 mm x 60 mm pieces. The resulting green labels were then fired in a conventional resistive furnace in air according to the following schedule:

(1) Room temperature to 400°C at 5°C/min.; and

(2) Held at 400°C for 0.4 hour.

The fired ceramic labels were placed onto hot cathode ray tube glasses (see Example 1) held at a temperature of 530°C in the resistive furnace. The labels were allowed to firmly bond to the cathode ray tube glasses before the furnace was turned off and the samples were allowed to cool down to room temperature. The ceramic labels were observed to be firmly bonded to the surface of the cathode ray tube glasses. The white top layers of the ceramic labels were then etched by a laser beam produced by a static CO_2 laser machine (available under the trade designation "MAC 2000 CO_2" from Dynamark Co., Bloomfield, CT) in order to expose a portion of the underlying black base layer and thereby form bar code patterns on the surfaces of ceramic labels.

Other embodiments of this invention will be apparent to those skilled in the art upon consideration of this specification or from practice of the invention disclosed herein. Various omissions, modifications, and changes to the principles and embodiments described herein may be made by one skilled in the art without

departing from the true scope and spirit of the invention which is indicated by the following claims.

What is claimed is:

1. A composite material suitable for labeling a substrate, comprising:

(a) a fired ceramic body comprising a base layer, the base layer including:

(i) a first glassy phase;

(ii) a first refractory phase interspersed with the first glassy phase, the first glassy phase being capable of wetting a substrate at an application temperature; and

(b) a top layer provided on the fired ceramic body,

wherein there is sufficient color contrast between the top layer and the fired ceramic body such that a code pattern is optically discernible, and wherein the composite material includes the code pattern.

2. The composite material of claim 1, wherein the code pattern comprises a bar code pattern.

3. The composite material of claim 1, wherein the application temperature is a temperature in the range from about 400°C to about 1000°C.

4. The composite material of claim 1, wherein the top layer consists essentially of a refractory material selected from the group consisting of a metal, an intermetallic material, a metal alloy, and combinations thereof.

5. The composite material of claim 1, wherein the top layer comprises a second glassy phase and a second refractory phase interspersed with the second glassy phase.

6. The composite material of claim 1, wherein the first refractory phase comprises a first plurality of refractory particles.

7. The composite material of claim 5, wherein the first and second glassy phases each has a first and second softening temperature, respectively, and wherein the second softening temperature is greater than the first softening temperature.

8. A labeled cathode ray tube, comprising:

(a) a cathode ray tube ceramic surface; and

(b) a composite material of any one of claims 1 – 7 attached to the cathode ray tube.

9. A method of applying a composite ceramic label to a substrate, comprising the steps of:

(a) providing a composite material of any one of claims 1 - 7;

(b) heating at least one of a ceramic substrate or the composite material to the application temperature; and

(c) after step (b), attaching the composite material to the substrate such that the first glassy phase bonds to the ceramic substrate.

10. A method for making a composite refractory barcode article, the method comprising the steps of:

(a) providing a ceramic composite suitable for labeling a substrate, comprising:

(i) a fired ceramic body comprising a base layer, the base layer including:

(1) a first glassy phase;

(2) a first refractory phase interspersed with the first glassy phase,

the first glassy phase being capable of wetting a substrate at an application temperature; and

(ii) a top layer provided on the fired ceramic body, and

(b) ablating by laser a portion of the top layer of the ceramic composite to providing a barcode,

wherein there is sufficient color contrast between the top layer and the fired ceramic body such that the bar code pattern is optically discernible.

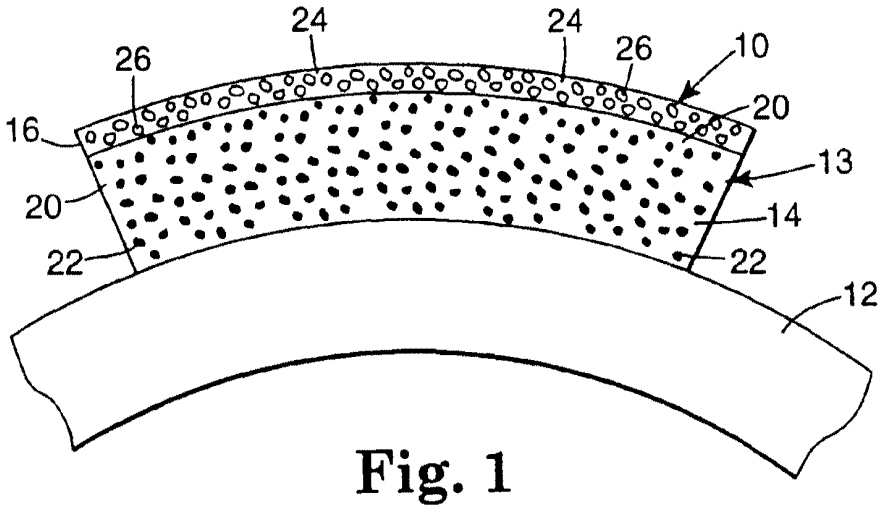


Fig. 1

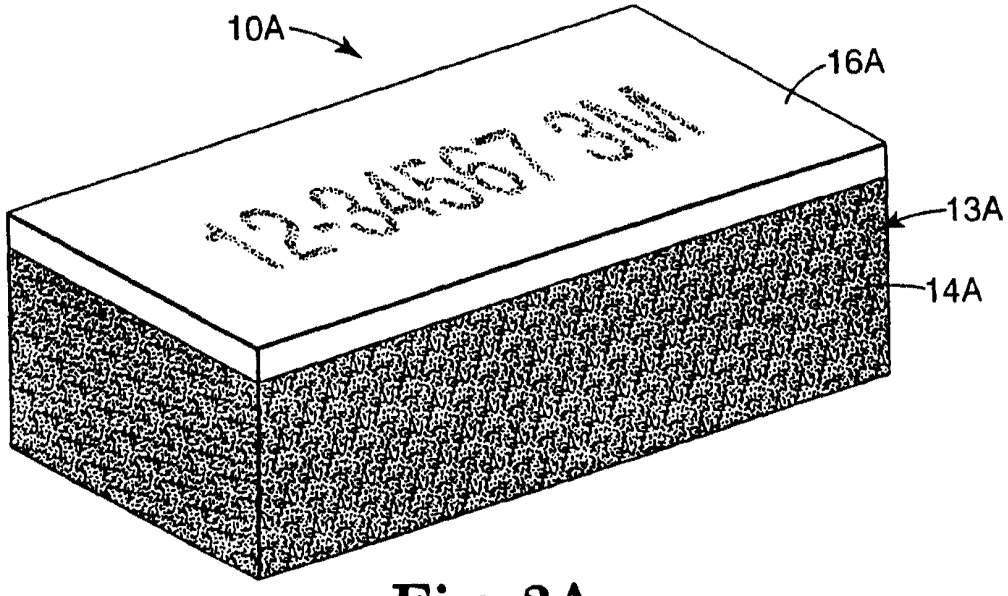


Fig. 2A

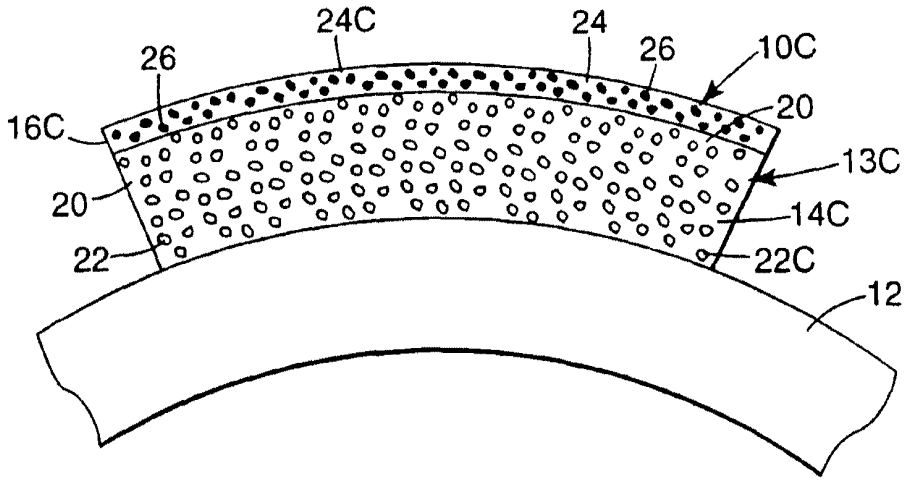


Fig. 3

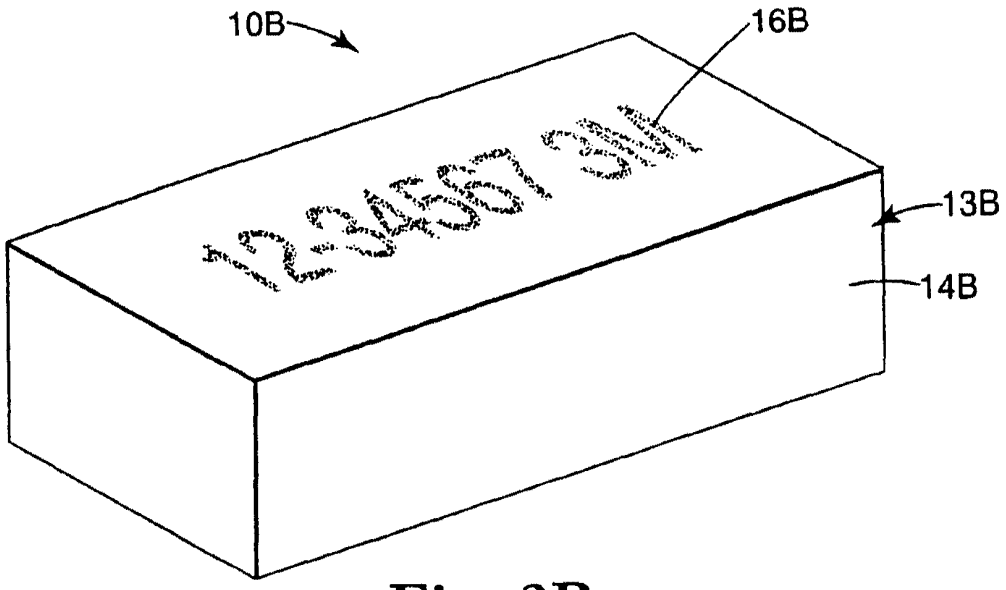


Fig. 2B

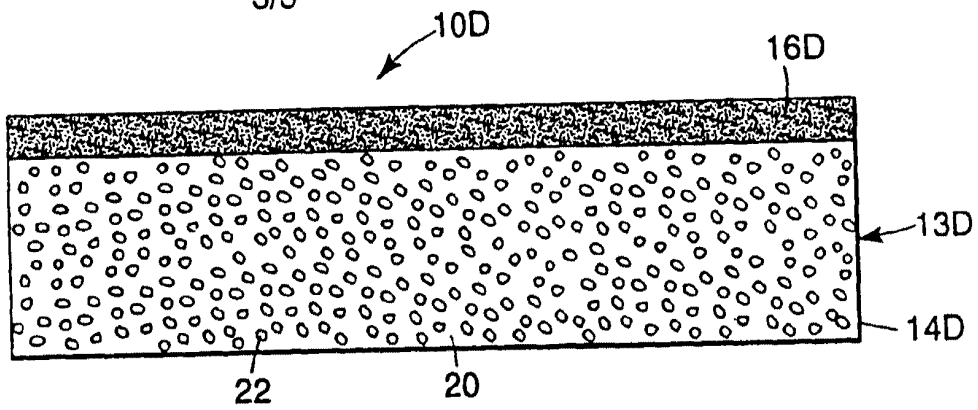


Fig. 4

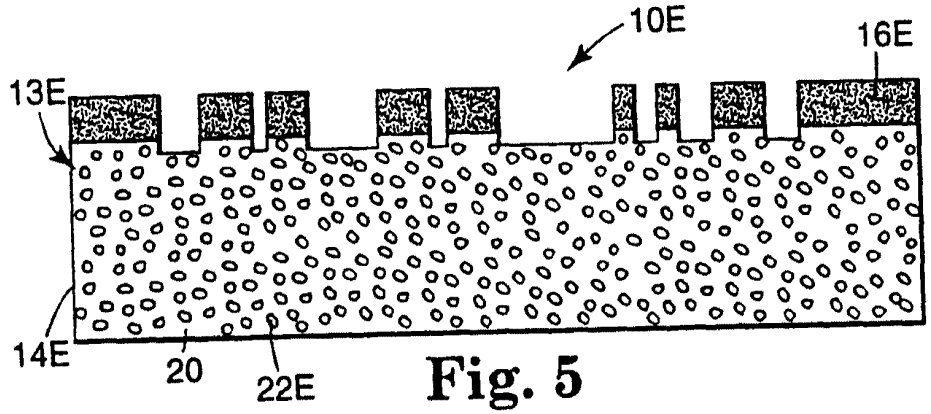


Fig. 5

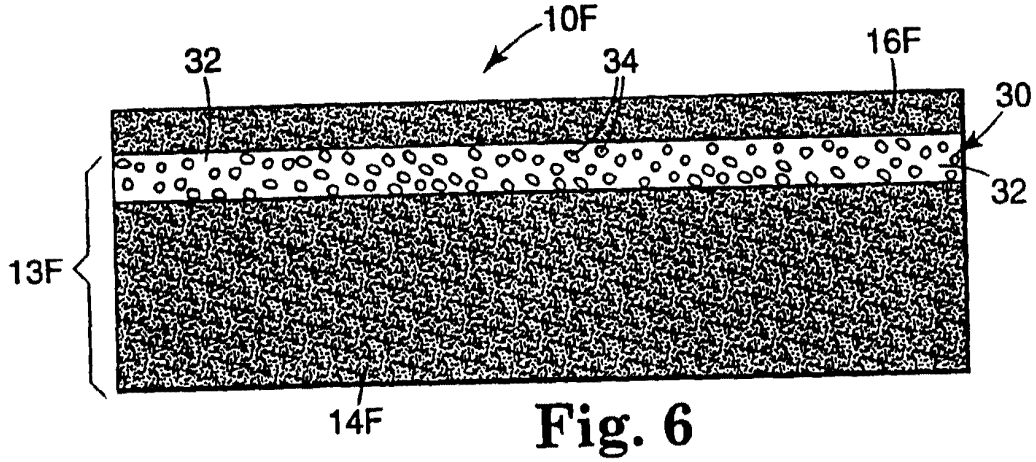


Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/10300

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G09F3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G09F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	US 5 362 554 A (HOLZER MARK R ET AL) 8 November 1994 see the whole document	1-3,9,10
A	WO 93 07844 A (W H BRADY CO) 29 April 1993 see page 1, line 1 - page 3, line 2	1,8
A	US 5 273 798 A (MINER AMOS J) 28 December 1993 see claim 1	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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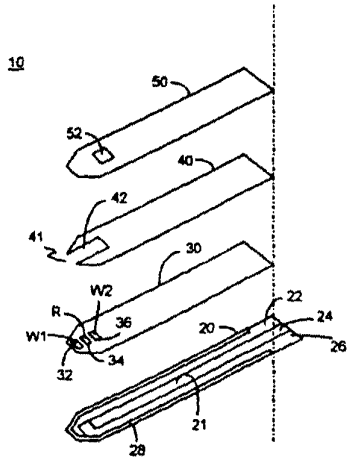
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(54) Title: DISPOSABLE SENSOR AND METHOD OF MAKING



(57) Abstract: A disposable electrode strip for testing a fluid sample including a laminated strip with a first and second end, a reference electrode embedded in the laminated strip proximate to the first end, at least two working electrodes embedded in the laminated strip proximate to the first end and the reference electrode, an open path for receiving a fluid sample beginning from the first end and being sufficiently long to expose the reference electrode and the working electrodes to the fluid sample, and conductive contacts located at the second end of the laminated strip. The laminated strip has a base layer with a conductive coating, a reagent holding layer, a channel forming layer and a cover. One of the working electrodes contains a reagent substantially similar to the reagent of the reference electrode and a second working electrode contains a reagent having an enzyme.

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DISPOSABLE SENSOR AND METHOD OF MAKING

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to electrochemical sensors that can be used for the quantification of a specific component or analyte in a liquid sample. Particularly, this invention relates to a new and improved electrochemical sensor and to a new and improved method of fabricating electrochemical sensors. More particularly, this invention relates to a disposable electrochemical sensor that is inexpensive to manufacture. Even more particularly, this invention relates to a disposable electrochemical sensor that gives accurate readings in the presence of interferants and varying red blood cells (hematocrit). Still even more particularly, this invention relates to disposable electrochemical sensors which are used for performing electrochemical assays for the accurate determination of analytes in physiological fluids.

2. Description of the Prior Art

Biosensors have been known for more than three decades. They are used to determine concentrations of various analytes in fluids. Of particular interest is the measurement of blood glucose. It is well known that the concentration of blood glucose is extremely important for maintaining homeostasis. Products that measure fluctuations in a person's blood sugar, or glucose levels have become everyday necessities for many of the nation's millions of diabetics. Because this disorder can cause dangerous anomalies in blood chemistry and is believed to be a contributor to vision loss and kidney failure, most diabetics need to test themselves periodically and adjust their glucose level accordingly, usually with insulin injections. If the concentration of blood glucose is below the normal range, patients can suffer from unconsciousness and lowered blood pressure which may even result in death. If the blood glucose concentration is higher than the normal range, the excess blood glucose can result in synthesis of fatty acids

and cholesterol, and in diabetics, coma. Thus, the measurement of blood glucose levels has become a daily necessity for diabetic individuals who control their level of blood glucose by insulin therapy.

Patients who are insulin dependent are instructed by doctors to check their blood-sugar levels as often as four times a day. To accommodate a normal life style to the need of frequent monitoring of glucose levels, home blood glucose testing was made available with the development of reagent strips for whole blood testing.

One type of blood glucose biosensors is an enzyme electrode combined with a mediator compound which shuttles electrons between the enzyme and the electrode resulting in a measurable current signal when glucose is present. The most commonly used mediators are potassium ferricyanide, ferrocene and its derivatives, as well as other metal-complexes. Many sensors based on this second type of electrode have been disclosed. Examples of this type of device are disclosed in the following patents.

U.S. Patent No. 5,628,890 (1997, Carter et al.) discloses an electrode strip having an electrode support, a reference or counter electrode disposed on the support, a working electrode spaced from the reference or counter electrode on the support, a covering layer defining an enclosed space over the reference and working electrodes and having an aperture for receiving a sample into the enclosed space, and a plurality of mesh layers interposed in the enclosed space between the covering layer and the support. The covering layer has a sample application aperture spaced from the electrodes. The working electrode includes an enzyme capable of catalyzing a reaction involving a substrate for the enzyme and a mediator capable of transferring electrons between the enzyme-catalyzed reaction and the working electrode.

This device proposes to reduce the effect of hematocrit on the sensor readings. According to the disclosure, this results from the downstream spacing of the reference electrode relative to the working electrode in combination with the thin layer of the sample solution created by the mesh layers.

U.S. Patent No. 5,708,247 (1998, McAleer et al.) discloses a disposable glucose test strip having a substrate, a reference electrode, a working electrode, and a means for making an electrical connection. The working electrode has a conductive base layer and a coating layer disposed over the conductive base layer. The coating layer is a filler having both hydrophobic and hydrophilic surface regions which form a network, an enzyme and a mediator.

U.S. Patent No. 5,682,884 (1997, Hill et al.) discloses a strip electrode with screen printing. The strip has an elongated support which includes a first and second conductor each extending along the support. An active electrode, positioned to contact the liquid mixture and the first conductor, has a deposit of an enzyme capable of catalyzing a reaction and an electron mediator. A reference electrode is positioned to contact the mixture and the second conductor.

U.S. Patent No. 5,759,364 (1998, Charlton et al.) discloses an electrochemical biosensor having an insulating base plate bearing an electrode on its surface which reacts with an analyte to produce mobile electrons. The base plate is mated with a lid of deformable material which has a concave area surrounded by a flat surface so that when mated to the base plate there is formed a capillary space into which a fluid test sample can be drawn. The side of the lid facing the base is coated with a polymeric material which serves to bond the lid to the base plate and to increase the hydrophilic nature of the capillary space.

U.S. Patent No. 5,762,770 (1998, Pritchard et al.) discloses an electrochemical biosensor test strip that has a minimum volume blood sample requirement of about 9 microliters. The test strip has a working and counter electrodes that are substantially the same size and made of the same electrically conducting material placed on a first insulating substrate. Overlaying the electrodes is a second insulating substrate which includes a cutout portion that forms a reagent well. The cutout portion exposes a smaller area of the counter electrode than the working electrode. A reagent for analysis of an analyte substantially covers the exposed areas of the working and counter electrodes in

the reagent well. Overlaying the reagent well and affixed to the second insulating substrate is a spreading mesh that is impregnated with a surfactant.

U.S. Patent No. 5,755,953 (1998, Henning et al.) discloses an reduced-interference biosensor. The device generally comprises an electrode used to electrochemically measure the concentration of an analyte of interest in a solution. The device includes a peroxidase enzyme covalently bound to microparticle carbon and retained in a matrix in intimate contact with the electrode. According to this disclosure, it is the enzyme/microparticle carbon of the device which provides a composition which is displays little sensitivity to known interfering substances.

U.S. Patent No. 5,120,420 (1992, Nankai et al.) discloses a biosensor with a base board having an electrode system mainly made of carbon, an insulating layer, a reaction layer containing an enzyme layer thereon, a spacer and a cover. The spacer creates a channel with an inlet and an outlet for holding a sample.

However, the prior art devices suffer from various shortcomings. One of these shortcomings is interference with biosensor readings caused by other substances in the sample fluid which can oxidize at the same potential. Prevalent among these are ascorbic acid, uric acid and acetaminophen. As these and other interfering substances oxidize, the current resulting from their oxidation is added to and indistinguishable from the current resulting from the oxidation of the blood analyte being measured. An error therefore results in the quantification of the blood analyte.

Another shortcoming is the interference caused by red blood cells (the hematocrit effect). This interference tends to cause an artificially high response rate for low hematocrit levels and, conversely, an artificially low response rate for high hematocrit levels.

Additional shortcomings of the prior art devices are that they have a more limited linear range and require a relatively large quantity of sample volume. Further, they require a relatively longer waiting time for development of a steady-state response before a reading can be achieved. Each of these shortcomings may, either individually or when combined with one or more of the other

shortcomings, contribute to erroneous measurement readings during analysis. Preliminary tests performed by the inventors of the present invention have shown that the prior art which claims to reduce the effect of hematocrit on glucose readings, were limited to and worked only in lower glucose concentrations.

Because of the importance of obtaining accurate glucose readings, it would be highly desirable to develop a reliable and user-friendly electrochemical sensor which does not have all of the drawbacks mentioned above. Therefore what is needed is an electrochemical sensor that incorporates an interference-correcting electrode to minimize the interference caused by oxidizable substances present in the sample fluid. What is further needed is an electrochemical sensor whose response is substantially independent of the hematocrit of the sample fluid. What is still further needed is an electrochemical sensor which requires less sample volume than previously required by the prior art. Yet, what is still further needed is an electrochemical sensor which has a wide linear measurement range; that is, a sensor having a reduced or negligible interference effect and useable over a wider glucose concentration.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an improved electrochemical sensor which combines an enzyme and a mediator. It is a further object of the present invention to provide an electrochemical sensor that incorporates an interference-correcting electrode to minimize the interference caused by oxidizable substances present in the sample fluid. It is a further object of the present invention to provide an electrochemical sensor whose response is substantially independent of the hematocrit levels of the sample fluid. It is still another object of the present invention to provide an electrochemical sensor which requires less sample volume than previously required by the prior art. It is yet another object of the present invention to provide an electrochemical sensor which has a wide linear measurement range.

The present invention achieves these and other objectives by providing an electrochemical sensor which requires a smaller sample size and compensates

for interference from oxidizable species in the sample and from varying hematocrit levels. The present invention has a laminated, elongated body having a sample fluid channel connected between an opening on one end of the laminated body and a vent hole spaced from the opening. Within the fluid channel lies at least two working electrodes and a reference electrode. The arrangement of the two working electrodes and the reference electrode is not important for purposes of the results obtained from the electrochemical sensor. The working electrodes and the reference electrode are each in electrical contact with separate conductive conduits, respectively. The separate conductive conduits terminate and are exposed for making an electrical connection to a reading device on the end opposite the open channel end of the laminated body.

The laminated body has a base insulating layer made from a plastic material. Several conductive conduits are delineated on the base insulating layer. The conductive conduits may be deposited on the insulating layer by screen printing, by vapor deposition, or by any method that provides for a conductive layer which adheres to the base insulating layer. The conductive conduits may be individually disposed on the insulating layer, or a conductive layer may be disposed on the insulating layer followed by etching/scribing the required number of conductive conduits. The etching process may be accomplished chemically, by mechanically scribing lines in the conductive layer, by using a laser to scribe the conductive layer into separate conductive conduits, or by any means that will cause a break between and among the separate conductive conduits required by the present invention. The preferred conductive coatings are gold film or a tin oxide/gold film composition. It should be pointed out that although the same electrically conducting substance (gold film or tin oxide/gold film) after scoring is used as conducting material for both working electrodes and the reference electrode, this material itself cannot function as a reference electrode. To make the reference electrode work, there must be a redox reaction (e.g., $Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$) at the electrically conducting material when a potential is applied. Therefore, a redox mediator must be present at the conducting material used for the reference electrode.

On top of the base insulating layer and the conductive conduits, the laminated body has a first middle insulating layer containing cutouts for at least two working electrodes and a reference electrode. One of the working electrodes and reference electrode may share the same cutout, provided that the electrode material (described later) disposed in the cutout is scored to isolate the working electrode from the reference electrode. Where three cutouts are used, each cutout corresponds to and exposes a small portion of a single conductive conduit. The cutouts for the working electrodes are substantially the same size. The cutout for the reference electrode may be the same or different size as the cutouts for the working electrodes. The placement of all of the cutouts are such that they will all co-exist within the sample fluid channel described above. This first middle insulating layer is also made of an insulating dielectric material, preferably plastic, and may be made by die cutting the material mechanically or with a laser and then fastening the material to the base layer. An adhesive, such as a pressure-sensitive adhesive, may be used to secure the first middle insulating layer to the base layer. Adhesion may also be accomplished by ultrasonically bonding the first middle layer to the base layer. The first middle insulating layer may also be made by screen printing the first middle insulating layer over the base layer.

The thickness of the first middle layer must be of sufficient thickness for loading a sufficient amount of electrode material for use as an electrochemical sensor. Each cutout contains electrode material. The electrode material has a redox mediator with at least one of a stabilizer, a binder, a surfactant, and a buffer. At least one of the cutouts also contains an enzyme capable of catalyzing a reaction involving a substrate for the enzyme. The redox mediator is capable of transferring electrons between the enzyme-catalyzed reaction and the working electrode.

The laminated body also has a second middle insulating layer on top of the first middle layer. The second middle layer is also made of a plastic insulating material and creates the sample fluid channel of the laminated body. It contains a U-shaped cutout on one end which overlays the cutouts on the first middle layer

with the open end corresponding to the open end of the laminated body described earlier.

The laminated body of the present invention has a top layer with a vent opening. The vent opening is located such that at least a portion of the vent opening overlays the bottom of the U-shaped cutout of the second middle insulating layer. The vent allows air within the sample fluid channel to escape as the sample fluid enters the open end of the laminated body. The sample fluid generally fills the sample fluid channel by capillary action. In small volume situations, the extent of capillary action is dependent on the hydrophobic/hydrophilic nature of the surfaces in contact with the fluid undergoing capillary action. This is also known as the wetability of the material. Capillary forces are enhanced by either using a hydrophilic insulating material to form the top layer, or by coating at least a portion of one side of a hydrophobic insulating material with a hydrophilic substance in the area of the top layer that faces the sample fluid channel between the open end of the laminated body and the vent opening of the top layer. It should be understood that an entire side of the top layer may be coated with the hydrophilic substance and then bonded to the second middle layer.

The insulating layers of the laminated body may be made from any dielectric material. The preferred material is a plastic material. Examples of acceptable compositions for use as the dielectric material are polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic, and polystyrene.

The number of cutouts in the first middle layer can be one, two and three or more. To use only one cutout, the single cutout must expose portions of two conductive conduits. The electrode material within the single cutout is scored in the middle to separate it into two parts; one acting as the working electrode and the other acting as the reference electrode. Such an arrangement allows for testing a smaller sample volume compared to two or three cutout embodiment.

However, this embodiment lacks the interference and hematocrit correction features of the other embodiments.

An embodiment having two cutouts is an alternative to the single cutout version. It has one cutout serving as the working electrode and the other one serving as a reference electrode. Another embodiment of the two cutout version combines the features of making the single cutout with that of the two cutout version. One of the cutouts containing electrode material is scored into two parts, one part serving as a first working electrode and the second part serving as the reference electrode. The second cutout serves as a second working electrode. Such a design is an alternative embodiment of the preferred embodiment of the present invention. This version of the two-cutout embodiment has the interference and hematocrit correction features but also allows for measuring an even smaller sample volume than that of the three-cutout embodiment.

In the three-cutout embodiment, two cutouts contain material for the working electrodes (W1 and W2) and one for the reference electrode (R). W2 further contains the enzyme capable of catalyzing a substrate of the enzyme. The three electrodes are positioned and sized in such a way that the resistance of the fluid sample could be precisely measured and the possible carry-over from W2 could be minimized. The possible electrode arrangements within the sample fluid channel may be W1-W2-R, W1-R-W2, R-W1-W2, W2-W1-R, W2-R-W1, or R-W2-W1 with the arrangement listed as the arrangement of electrodes would appear from the open end of the laminated body to the vent opening. The preferred position was found to be W1-R -W2; that is, as the sample fluid entered the open end of the laminated body, the fluid would cover W1 first, then R, then W2. The preferred position allows for the precise measurement of blood sample resistance. This is necessary for good correlation between the resistance and hematocrit level in the blood sample.

As mentioned earlier, oxidizable interferants such as ascorbic acid, uric acid and acetaminophen, to name a few, cause inaccurate readings in the output of an electrochemical biosensor. The present invention negates this effect by subtracting the current response at W1 (first working electrode) from the current

response from W2 (second working electrode) to calculate the enzyme concentration in the sample fluid. This is achieved by maintaining the surface area of W1 substantially equal to the surface area of W2. Also important is the composition of the reagents disposed on W1 and W2. The reagents are designed to have a minimal effect on the response of the interferences which also contributes to the accuracy of the analyte measurement.

The hematocrit interference is reduced by using a two-step process. First, the resistance (r-value) between W1 (first working electrode) and R (reference electrode) is measured. The r-value is then used to estimate the hematocrit level in the sample fluid. The following equation represents this relationship:

$$r = k_1 / (1-H) \qquad \text{Eq. (1)}$$

where r is resistance value measured in Ohms or Kilo-Ohms
H is hematocrit level
k_1 is a constant equal to 4.6 (r measured in Kilo-Ohms)

Second, the hematocrit level value is then used to mathematically correct the enzyme concentration reading obtained from above. The following equation represents the calculation performed using the calculated hematocrit level from Eq. (1):

$$C_{corr} = C_{mea} / (k_2+k_3C_{mea}+(k_4+k_5C_{mea})(1-H))$$

Eq. (2)

where C_{corr} is the corrected analyte concentration
C_{mea} is the measured analyte concentration
k_2 is a constant equal to 1.03
k_3 is a constant equal to -0.003
k_4 is a constant equal to -0.1
k_5 is a constant equal to 0.0054

H is the calculated hematocrit level from Eq. (1)

The constant values above have been determined for the preferred embodiment of the present invention. Varying the surface area of the electrode areas and the formulations of the reagents may require one skilled in the art to calculate new values for constants k_1-k_5 in order to more accurately determine corrected glucose concentration.

All of the advantages of the present invention will be made clearer upon review of the detailed description, drawings and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a perspective view of the present invention showing the open end, the vent and the electrical contact points of the laminated body.

FIGURE 2 is an exploded, perspective view of the present invention showing the various layers of the laminated body.

FIGURES 3A, 3B, 3C, and 3D are top views of a strip of each layer of the present invention showing the patterns for making multiple sensors of the present invention.

FIGURE 3E is a top view of a segment of the laminated strip of the present invention showing the patterns for making multiple sensors of the present invention.

FIGURES 4A and 4B are graphs showing the effect of hematocrit on the concentration response of the present invention in normal and high concentrations of blood glucose.

FIGURE 5 is a correlation of sample volume on the concentration response of the present invention.

FIGURE 6 is a correlation curve of the concentration readings using sensors of the present invention versus the concentration readings of obtained on the same samples using a YSI glucose analyzer.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The preferred embodiment of the present invention is illustrated in FIGURES 1-6 . Figure 1 shows a sensor **10** of the present invention. Sensor **10** has a laminated body **100**, a fluid sampling end **110**, an electrical contact end **120**, and a vent opening **52**. Fluid sampling end **110** includes a sample fluid channel **112** between a sampling end aperture **114** and vent opening **52**. Electrical contact end **120** has at least three discreet conductive contacts **122**, **124** and **126**.

Referring now to Figure 2, laminated body **100** is composed of a base insulating layer **20**, a first middle layer **30**, a second middle layer **40**, and a top layer **50**. All layers are made of a dielectric material, preferably plastic. Examples of a preferred dielectric material are polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic and polystyrene. Base insulating layer **20** has a conductive layer **21** on which is delineated a first conductive conduit **22**, a second conductive conduit **24** and a third conductive conduit **26**. Conductive conduits **22**, **24** and **26** may be formed by scribing or scoring the conductive layer **21** as illustrated in Fig. 2 or by silk-screening the conductive conduits **22**, **24** and **26** onto base layer **20**. Scribing or scoring of conductive layer **21** may be done by mechanically scribing the conductive layer **21** sufficiently to create the three independent conductive conduits **22**, **24** and **26**. The preferred scribing or scoring method of the present invention is done by using a carbon dioxide (CO_2) laser, a YAG laser or an eximer laser. An additional scoring line **28** (enlarged and not to scale; for illustrative purposes only) may be

made, but is not necessary to the functionality of sensor **10**, along the outer edge of base layer **20** in order to avoid potential static problems which could give rise to a noisy signal. Conductive layer **21** may be made of any electrically conductive material, preferably gold or tin oxide/gold. A useable material for base layer **20** is a tin oxide/gold polyester film (Cat. No. FM-1) or a gold polyester film (Cat. No. FM-2) sold by Courtaulds Performance Films, Canoga Park, California.

First middle layer **30** has a first electrode cutout **32** which exposes a portion of first conductive conduit **22**, a second electrode cutout **34** which exposes a portion of second conductive conduit **24** and a third electrode cutout **36** which exposes a portion of third conductive conduit **26**. First layer **30** is made of a plastic material, preferably a medical grade one-sided tape available from Adhesive Research, Inc., of Glen Rock, Pennsylvania. Acceptable thickness of the tape for use in the present invention are in the range of about 0.003 in. (0.76 mm) to about 0.005 in. (0.127 mm). One such tape, Arcare® 7815, was preferred because of its ease of handling and it showed good performance in terms of its ability to hold a sufficient quantity of chemical reagents and to promote a favorable blood flood speed (capillary action) through sample fluid channel **112** of sensor **10**. It should be understood that the use of a tape is not required. A plastic insulating layer may be coated with a pressure sensitive adhesive, or may be ultrasonically-bonded to base layer **20**, or may be silk-screened onto base layer **20** to achieve the same results as using the polyester tape mentioned.

The three cutouts **32**, **34** and **36** define electrode areas **W1**, **R** and **W2**, respectively, and hold chemical reagents forming two working electrodes and one reference electrode. Typically, electrode area **R** must be loaded with a redox reagent or mediator to make the reference electrode function. If **R** is not loaded with a redox reagent or mediator, working electrodes **W1** and **W2** will not work. Electrode areas **W1** and **R** are loaded preferably with the same chemical reagent to facilitate the resistance measurement described earlier. The reagents preferably contain an oxidized form of a redox mediator, a stabilizer, a binder, a

surfactant, and a buffer. Typically, the redox mediator may be at least one of ferrocene, potassium ferricyanide and other ferrocene derivatives. The preferred stabilizer is polyethylene glycol, the preferred binder is methyl cellulose, the preferred surfactant is t-octylphenoxypolyethoxyethanol, and the preferred buffer is a citrate buffer. Electrode area **W2** is preferably loaded with the same chemical reagents loaded into electrode areas **W1** and **R** but with the addition of an enzyme capable of catalyzing a reaction involving a substrate for the enzyme or a substrate catalytically reactive with an enzyme and a mediator capable of transferring electrons transferred between the enzyme-catalyzed reaction and the working electrode to create a current representative of the activity of the enzyme or substrate and representative of the compound.

The cutouts and electrode areas of first layer **30** are positioned relative to each other and to the flow of the sample fluid in sample fluid channel **112** such that the resistance of the sample fluid may be precisely measured and the possible carryover from electrode area **W2** to electrode area **W1** could be minimized. Using fluid sample end **110** of sensor **10** as a reference point, the arrangements of the electrode areas could be **W1-W2-R**, **W1-R-W2**, **R-W1-W2**, **W2-W1-R**, **W2-R-W1**, or **R-W2-W1**. The preferred position was found to be **W1-R-W2**.

Second middle layer **40** has a U-shaped channel cutout **42** located at second layer sensor end **41**. The length of channel cutout **42** is such that when second middle layer **40** is layered on top of first middle layer **30**, electrode areas **W1**, **W2** and **R** are within the space defined by channel cutout **42**. The thickness of second middle layer **40** was found to be critical for the speed of the sample fluid flow into sample fluid channel **112,** which is filled by capillary action of the sample fluid.

Top layer **50**, which is placed over second middle layer **40**, has a vent opening **52** spaced from fluid sample end **110** of sensor **10** to insure that sample fluid in fluid channel **112** will completely cover electrode areas **W1**, **W2** and **R**. Vent opening **52** is placed in top layer **50** so that it will align somewhat with the

bottom of channel cutout **42** of second middle layer **40**. Preferably, vent opening **52** will expose a portion of and partially overlay the bottom of the U-shaped cutout **42** of second middle layer **40**.

Preparation of Reagents 1 & 2

Reagents 1 and 2 comprise the oxidized form of a redox mediator, a stabilizer, a binder, a surfactant, and a buffer. Reagent 2, in addition, contains an enzyme. The oxidized form of the redox mediator, potassium ferricyanide, was found to be stable in the matrices. The quantity used in the formulation must be sufficient to attain a workable linear range. The enzyme must also have sufficient activity, purity and stability. A commercially available glucose oxidase may be obtained from Biozyme, San Diego, California as Cat. No. G03A, about 270U/mg. The stabilizer must be sufficiently water-soluble and be capable of stabilizing both the mediator and the enzyme. The binder should also be capable of binding all other chemicals in the reagents in electrode areas **W1**, **W2** and **R** to the conductive surface/layer **21** of base layer **20**. The preferred stabilizer is polyethylene glycol (Cat. No. P4338, Sigma Chemicals, St. Louis, MO). The preferred binder is Methocel 60 HG (Cat. No. 64655, Fluka Chemical, Milwaukee, WI). The buffer solution must have sufficient buffer capacity and pH value to optimize the enzyme reaction. A 0.05M citrate buffer is preferred. The surfactant is necessary to facilitate dispensing of Reagents 1 and 2 into cutouts **32**, **34** and **36** of middle layer **30** as well as for quickly dissolving the dry chemical reagents. The amount and type of surfactant is selected to assure the previously mentioned functions and to avoid a denaturing effect on the enzyme. The preferred surfactant is Triton X-100. The reagents are prepared as follows:

Reagent 1

Step 1: Prepare 50 mM citrate buffer (pH 5.7) by dissolving 0.1512 grams citric acid and 1.2580 grams sodium citrate in 100 ml of deionized water.

Step 2: Prepare a 1% methocel 60HG solution by stirring 1 gram of methocel in 100 ml of citrate buffer from Step 1 for 12 hours.

Step 3. Add 0.3 ml of 10% Triton X-100 into the methocel solution

Step 4. Add 2.5 grams of polyethylene glycol into the solution from Step 3.

Step 5: While stirring, add 1 gram of potassium ferricyanide to the solution from Step 4.

Reagent 2

Step 1-Step 4: same steps as Reagent 1.

Step 5: While stirring, add 6.5 grams potassium ferricyanide to the solution of Step 4.

Step 6: Add 1.0 gram of glucose oxidase to the solution of Step 5 and stir for 10 minutes or until all solid materials are completely dissolved.

Electrode Construction

A piece of a gold or tin oxide/gold polyester film available from Courtaulds Performance Films is cut to shape, as illustrated in Fig. 2, forming base layer **20** of sensor **10**. A CO_2 laser was used to score the gold or tin oxide/gold polyester film. As illustrated in Fig. 2, the film was scored by the laser such that three electrodes at sample fluid end **110** and three contact points **122**, **124** and **126** were formed at electrical contact end **120**. The scoring line is very thin but sufficient to create three separate electrical conductors. A scoring line **28** can be made, but is not necessary, along the outer edge of base layer **20** to avoid potential static problems which could cause a noisy signal from the finished sensor **10**.

A piece of one-sided adhesive tape is then cut to size and shape forming first middle layer **30** so that it will cover a majority of the conductive layer **21** of base layer **20** except for exposing a small electrical contact area illustrated in Fig. 1. Three rectangular, square or circular cutouts **32**, **34** and **36** of substantially equal size are punched by CO_2 laser (25W laser available from Synrad, Inc., San Diego, CA). Cutouts **32**, **34** and **36** define the electrode areas **W1**, **W2** and **R** which hold chemical reagents. The size of the cutouts is preferred to be made as small as possible in order to make the fluid sample channel **112** of sensor **10** as

short as possible while still being capable of holding sufficient chemical reagent to function properly. The preferred hole size for the present invention has a typical dimension of about 0.033 in. (0.84 mm) by about 0.043 in. (1.09 mm). As illustrated in Fig. 2, cutouts **32**, **34** and **36** are aligned with each other and having a spacing of about 0.028 in. (0.71 mm) between them. The rectangular cutouts are for illustrative purposes only. It should be understood that the shape of the cutouts is not critical provided that the size of the cutouts is big enough to hold sufficient chemical reagents for the electrodes to function properly but small enough to allow for a reasonably small sample channel. As noted earlier, changing the shape of the cutouts or the surface area of the cutouts may require changing the constant values k_1-k_5 for Eq. 1 and Eq. 2. As stated previously, the preferred arrangement of the electrodes formed in cutouts **32**, **34** and **36** is **W1** (working electrode 1), **R** (reference electrode) and **W2** (working electrode 2).

0.4 microliters of Reagent 1 is dispensed into electrode areas **W1** and **R**. Reagent 1 is a mixture of a redox mediator, a stabilizer, a binder, a surfactant, and a buffer. The preferred mixture for Reagent 1 is made by mixing the following components in the described percentages (W/W%): about 1% potassium ferricyanide, about 2.5% polyethylene glycol, about 1% methocel 60 HG, about 0.03% Triton X-100 and about 0.05M citrate buffer (pH 5.7). 0.4 microliters of Reagent 2 is dispensed into electrode area **W2**. Reagent 2 is a mixture similar to that of Reagent 1 but with the addition of an enzyme capable of catalyzing a reaction involving a substrate of the enzyme. The preferred enzyme is glucose oxidase. The preferred mixture for Reagent 2 is made by mixing the following percentages (W/W%) of the following ingredients: about 6.5% potassium ferricyanide, about 2.5% polyethylene glycol, about 1% methocel 60 HG, about 0.03% Triton X-100, about 0.05M citrate buffer (pH 5.7), and about 1% glucose oxidase. After the addition of the reagents, the device was dried for about 2 minutes at 55°C in an oven. After drying, a piece of double-sided tape available from Adhesive Research was fashioned into second middle layer **40** with U-shaped channel **42**. Second middle layer **40** is then layered onto first middle layer **30**. As mentioned earlier, this second middle layer **40** serves as a

spacer and defines the size of the fluid sample channel **112**. Its width and length is optimized to provide for a relatively quick moving fluid sample. The preferred size of U-shaped channel **42** is about 0.063 in. (1.60 mm) wide by about 0.248 in. (6.30 mm) long.

A piece of a transparency film (Cat. No. PP2200 or PP2500 available from 3M) is fashioned into top layer **50**. A rectangular vent hole **52** is made using the CO_2 laser previously mentioned. The preferred size of vent hole **42** is about 0.075 in. (1.91 mm) by about 0.059 in. (1.50 mm). Vent hole **52** is located approximately 0.130 in. (3.3 mm) from fluid end **110** of sensor **10**. Top layer **50** is aligned and layered onto second middle layer **40** to complete the assembly, as illustrated in Fig. 1, of sensor **10**.

Although the description of electrode construction above describes construction for a single sensor, the design and materials used are ideal for making multiple sensors from one piece of each layer material as shown in Fig. 3A-3E. This would be accomplished by starting with a relative large piece of base layer **20** having conducting layer **21** thereon. A plurality of scored lines are made into conductive layer **21** such that a repetitive pattern, as illustrated in Fig. 3A, is created using the preferred scribing method described previously whereby each pattern will eventually define the three conductive paths **22**, **24** and **26** for each sensor. Similarly, a large piece of first middle layer **30**, which is illustrated in Fig. 3B and which also has a plurality of cutouts **32**, **34**, and **36** in a repetitive pattern, is sized to fit over base layer **20** in such a way that a plurality of sensors **10** will be had when completed. The size of each cutout and the electrode material disposed in the plurality of electrode areas **W1**, **R** and **W2** are similar to that disclosed above. After disposing Reagents 1 & 2 in their respective cutouts and dried, a large piece of second middle layer **40** having a plurality of elongated cutouts **42** and illustrated in Fig. 3C is layered onto first middle layer **30** such that each elongated cutout **42** of second middle layer **40** contains corresponding cutouts **32**, **34** and **36** of first middle layer **30**. A comparably-sized top layer **50** having a plurality of vent openings **52** in a repetitive pattern, as shown in Fig. 3D,

is layered onto second middle layer **40**. Fig. 3E is a top view of the combined layers. The laminated strip created by the four layers **20**, **30**, **40** and **50** has a plurality of sensors **10** that can be cut from the laminated strip. The laminated strip is cut longitudinally along line A-A' at fluid sampling end **210** to form a plurality of sampling apertures **114** and longitudinally along line B-B' at electrical contact end **220** to form a plurality of conductive contacts **122**, **124** and **126**. The laminated strip is also cut at predetermined intervals along line C-C' forming a plurality of individual sensors **10**. Shaping of the fluid sampling end **120** of each sensor **10**, as illustrated in Fig. 1, may be performed if desired. It should be understood by those skilled in the art that the order in which the laminated strip can be cut is not important. For instance, the laminated strip may be cut at the predetermined intervals (C-C') and then the cuts along A-A' and B-B' can be made to complete the process.

The following examples illustrate the unique features of the present invention which includes the compensation for varying hemotacrit levels by measuring sample fluid resistance and nullification of the interference effects of oxidizable species present in the sample fluid. All sensors of the present invention were tested on a breadboard glucose meter manufactured by Nova Biomedical Corporation of Waltham, MA. A potential of 0.35 Volts was applied across the working electrodes and the reference electrode and the resultant current signals were converted to glucose concentrations in accordance with the disclosure of the present invention. The readings were compared to readings (control readings) obtained on the same samples using YSI Glucose Analyzer (Model 2300) available from Yellow Springs Instruments, Inc., Yellow Springs, OH.

Example 1

Demonstration of Hematocrit Compensation

The unique design of the present invention makes it possible to measure the resistance of the fluid sample. This is achieved by applying the same reagent, Reagent 1, to the reference electrode **R** and the first working electrode

W1. The chemical reagents used in Reagent 1 are critical for accurate measurement of the resistance. Reagent 1 can not contain a large amount of salts or any glucose oxidase. Otherwise, the resulting resistance would not be accurate and would be glucose dependent. For proper functioning of the present invention, it should be noted that a minimum amount of a mediator such as potassium ferricyanide for the reference electrode is essential.

Resistance of a sample fluid, in this case blood samples, between **W1** and **R** is measured at any time, preferably 20 seconds after a reading device (Nova glucose meter) is triggered by the blood samples. Blood samples with different hematocrit levels were prepared by spinning a whole blood sample and recombining plasma and red blood cells in varying ratios. Hematocrit levels were measured with a micro hematocrit centrifuge. Concentrations of glucose in the various samples were measured by sensors of the present invention (C_{mea}) and by a YSI blood glucose analyzer (the control), Model 2300, Yellow Springs Instruments, Inc., Yellow Springs, OH. Equations (1) and (2), previously mentioned, were used to calculate the corrected glucose concentration (C_{corr}) measured by sensors of the present invention to demonstrate the hemotacrit compensation feature of the present invention. The data obtained was plotted and Figs. 4A and 4B show two graphs representing the percent correlation of the readings obtained using sensors of the present invention with the Nova glucose meter to the readings obtained for the samples using the YSI blood glucose analyzer at low and high levels of glucose in samples with varying hematocrit levels.

Example 2

Demonstration of Interference Free Feature

The unique design of the present invention makes it possible to eliminate interference from oxidizable substances such as ascorbic acid, acetaminophen, uric acid, and other possible interferants present in the sample. This is achieved by subtracting the response obtained from **W1** from the response obtained at **W2**, and is represented by the following equation:

$$I = I_{w_2} - I_{w_1} \qquad \text{Eq. (3)}$$

where I_{w_2} is the current at W2 (second working electrode)

I_{w_1} is the current at W1 (first working electrode)

I is the difference between W2 and W1 and represents the current due to oxidation of the mediator of its reduced form, which is proportional to the glucose concentration in the sample

Because W1 and W2 have the same surface area, the potential interference present in the sample fluid should give relatively identical signals from each working electrode. Even though W1 and W2 had different reagents, it was found that there was no remarkable difference in the response to the interference. Thus, the difference in current response obtained in blood samples was due to the glucose present in the samples. This was tested by spiking normal and high glucose blood samples with 1 mM and 5 mM ascorbic acid, acetaminophen and uric acid. Table 1 shows the percentage response change of the readings obtained with sensors of the present invention and various commercially available sensors (referred to as Strip 1, Strip 2, Strip 3, and Strip 4) in blood samples having a concentration of 100 mg/dL glucose and 300 mg/dL upon addition of the interferents.

Table 1 - Response change (%) Upon Addition of Interferent

100 mg/dL glucose : ascorbic acid					
mM	**Nova**	**Strip 1**	**Strip 2**	**Strip 3**	**Strip 4**
0	0	0	0	0	0
1	4	26.7	19.8	21.3	6

5	4.5	133.6	115.8	151.5	Error
300 mg/dL glucose : ascorbic acid					
0	0	0	0	0	0
1	-0.9	7.2	14	14.7	-4.3
5	-0.5	Hi	107.3	Hi	Error
100 mg/dL glucose : acetaminophen					
0	0	0	0	0	0
1	3.0	34.7	5.7	50	-8.2
5	3.4	90.8	38	136	-13.4
300 mg/dL glucose : acetaminophen					
0	0	0	0	0	0
1	-3.4	20	3.5	18	-7.2
5	-3.5	39	8.0	23.7	-17.5
100 mg/dL glucose : uric acid					
0	0	0	0	0	0
1	-2.7	32	35	35	-1.3
300 mg/dL glucose : uric acid					
0	0	0	0	0	0
1	-4.5	10	15	7	-1

From the test data, one observes that the readings obtained from sensors of the present invention show essentially no change in the presence of 1 mM and 5 mM ascorbic acid and acetaminophen, and 1 mM uric acid. All commercially available sensors except one, Strip 4, suffer from serious interference. Strip 4 showed an "error" for 5 mM ascorbic acid. At concentrations of 300 mg/dL

glucose, sensors of the present invention also showed no interference (response change of less than 5%) upon spiking the samples with 1 mM and 5 mM ascorbic acid. The commercially available sensors showed about 7% to about 15% response increase for 1 mM ascorbic acid spiked samples, and showed a "Hi" reading for 5 mM ascorbic acid spiked samples. Strip 4 again showed an "error" for 5 mM ascorbic acid spiked samples. In samples containing acetaminophen and uric acid, all commercially available strips showed varying degrees of error except for Strip 4 in samples containing uric acid.

Example 3

Demonstration of Minimum Sample Volumes Feature

The unique design of the present invention enables the measurement of sample sizes smaller than which have heretofore been possible. Blood samples are applied to the sensors and the samples travel along the fluid sample channel to the venting hole. The blood volume required for measurement of blood glucose is determined by the channel volume. The calculated volume for the present invention is 1.44 microliters. In order to test the volume effect on sensor response, different blood sample volumes were applied to the sensors and the resulting concentration readings were plotted against volume. The test data is shown in Fig. 5.

Sensors of the present invention show no dependence of the response on the sample volume if the volume is above 1.5 microliters. It was found that sensors of the present invention still gave reasonable readings on sample sizes as low as 1.0 microliters. This is possible because the hydrophilic character of Reagent 1 applied to W1 and R, and Reagent 2 applied to W2 permitted the sample to cover the electrode areas even though the blood volume did not fill the entire sample channel.

Example 4

Demonstration of Wide Linear Range and Precision Feature

A sample of venous blood was collected and separated into several aliquots. Each aliquot was spiked with different glucose concentrations ranging from 35 to 1000 mg/dL. The aliquots were each measured with a YSI glucose analyzer and then with sensors of the present invention using the Nova glucose meter. Sensors of the present invention show a linear relationship of current response vs. glucose concentration from 35 to 1000 mg/dL. The concentration readings were plotted against the concentration values obtained using the YSI meter (the control) and are illustrated in Fig. 6.

A regression coefficient of 0.9988 indicated a near perfect match with the readings obtained with the YSI blood glucose analyzer. The same aliquots were tested using four different commercially-available sensors with their accompanying meters. The commercially-available sensors showed a linear response only up to about 600 mg/dL. Above the 500-600 mg/dL range, all commercially available sensors displayed "Hi" as the test result.

The precision of the sensors of the present invention was investigated at the same glucose level range from about 35 to 1000 mg/dL. Four different batches of sensors of the present invention were used in the precision tests. Typically, the relative standard deviation was about 9.5%, 5.0%, 3.5%, 2.9%, and 2.6% for samples containing 35, 100, 200, 500, and 1000 mg/dL levels of glucose, respectively.

What is claimed is:

1. A disposable electrode strip for testing a fluid sample comprising:

a laminated strip having a first strip end, a second strip end and a vent opening spaced from said first strip end, said laminated strip comprising a base layer with at least three electrodes delineated thereon, a reagent holding layer carried on said base layer, said reagent holding layer having at least two cutouts, a channel forming layer carried on said reagent holding layer, and a cover;

an enclosed channel between said first strip end and said vent opening, said enclosed channel containing said at least two cutouts;

a first reagent disposed in a first cutout of said at least two cutouts forming a reference electrode;

a second reagent disposed in a second cutout of said at least two cutouts forming a first working electrode, said second reagent being substantially similar to said first reagent and containing an enzyme; and

conductive contacts at said second strip end and insulated from said enclosed channel.

2. The electrode strip of Claim 1 further comprising:

a third cutout in said reagent holding layer and contained within said enclosed channel; and

a third reagent disposed in said third cutout forming a second working electrode, said third reagent being substantially similar to said first reagent.

3. The electrode strip of Claim 2 wherein said first reagent, said second reagent and said third reagent contain a redox mediator.

4. The electrode strip of Claim 3 wherein said redox mediator is at least one metal complex.

5. The electrode strip of Claim 3 wherein said at least one redox mediator is potassium ferricyanide and other inorganic and organic redox mediators.

6. The electrode strip of Claim 1 wherein said base layer has a conductive coating disposed thereon for forming said at least two electrodes.

7. The electrode strip of Claim 6 wherein said conductive coating is gold.

8. The electrode strip of Claim 6 wherein said conductive coating comprising gold and tin oxide.

9. The electrode strip of Claim 6 wherein said base layer, said reagent holding layer, said channel forming layer, and said cover are made of a plastic dielectric material.

10. The electrode strip of Claim 9 wherein said plastic material is selected from the group consisting of polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic, and polystyrene.

11. The electrode strip of Claim 1 wherein said enclosed channel is hydrophilic.

12. The electrode strip of Claim 1 wherein said enclosed channel has a volume of about 1.44 microliters.

13. The electrode strip of Claim 1 wherein said cover has a hydrophilic coating on at least one side.

14. The electrode strip of Claim 3 wherein said first reagent, said second reagent and said third reagent further contain at least one of a stabilizer, a binder, a surfactant, and a buffer.

15. The electrode strip of Claim 14 wherein said stabilizer is a polyalkylene glycol, said binder is a cellulose material, and said surfactant is a polyoxyethylene ether.

16. The electrode strip of Claim 15 wherein said stabilizer is polyethylene glycol, said binder is methyl cellulose, said surfactant is t-octylphenoxypolyethoxyethanol, and said buffer is a citrate buffer.

17. The electrode strip of Claim 14 wherein said first reagent, said second reagent and said third reagent are made from a mixture having starting components comprising about 1wt% to about 6.5wt% of said redox mediator, about 2.5wt% of said stabilizer, about 1wt% of said binder, and about .03wt% of said surfactant in said buffer.

18. The electrode strip of Claim 17 wherein said citrate buffer is about 0.05M.

19. The electrode strip of Claim 1 wherein said channel forming layer has a thickness sufficient to optimize the flow of said fluid sample along said open path.

20. The electrode strip of Claim 19 wherein said thickness is about 0.007 inches (0.1778 mm).

21. The electrode strip of Claim 16 wherein said first reagent and said second reagent are made of a mixture having starting components comprising about 1wt% of said potassium ferricyanide, about 2.5wt% of said polyethylene

glycol, about 1wt% of said methyl cellulose, about .03wt% of said t-octylphenoxypolyethoxyethanol, and said citrate buffer is about 0.05M.

22. The electrode strip of Claim 16 wherein said third reagent is made of a mixture having starting components comprising about 6.5wt% of said potassium ferricyanide, about 2.5wt% of said polyethylene glycol, about 1wt% of said methyl cellulose, about .03wt% of said t-octylphenoxypolyethoxyethanol, and said pH buffer is about a 0.05M citrate buffer, and about 1wt% of said enzyme.

23. The electrode strip of Claim 22 wherein said enzyme is glucose oxidase.

24. The electrode strip of Claim 2 wherein the surface area of said first working electrode is substantially same as the surface area of said second working electrode.

25. A method of using an electrode strip for determining the concentration of an analyte, said electrode strip having a first working electrode, a second working electrode and a reference electrode wherein said first working electrode contains an enzyme capable of catalyzing a reaction involving a substrate for the enzyme, said first working electrode, said second working electrode and said reference electrode being disposed in a fluid sample channel for measuring a fluid sample, said method comprising:

disposing said fluid sample into said channel of said electrode strip;

applying a potential between said reference electrode and said first working electrode which contains said enzyme;

measuring a first current generated between said first working electrode and said reference electrode and correlating said first current to a concentration of said analyte in said fluid sample;

measuring a resistance value of said fluid sample between said second working electrode and said reference electrode;

applying said resistance value to a first equation and determining the hematocrit level of said fluid sample; and

calculating a corrected concentration of said analyte using a second equation to correct for the presence of hematocrit in said sample.

26. The method of Claim 25 wherein said method further comprising:

applying a potential between said reference electrode and said second working electrode;

measuring a second current generated between said second working electrode and said reference electrode;

subtracting said second current from said first current and obtaining a current difference, correlating said current difference to a concentration of said analyte in said fluid sample.

27. The method of Claim 26 wherein said method further includes triggering said current measuring step when said fluid sample contacts said first working electrode, said second working electrode and said reference electrode creating said first current and said second current.

28. The method of Claim 27 wherein said method further includes reading a current value for each of said first current and said second current at about a time where said current values for each of said first current and said second current reach a steady-state.

29. The method of Claim 28 wherein said reading is taken at about 20 seconds after said current measuring step is triggered.

30. A disposable electrode strip for detecting or measuring the concentration of at least one analyte in a fluid sample, said electrode strip comprising:

an insulating base strip having a first base end and a second base end;

a conductive layer disposed on one side of said base strip and scribed to delineate at least three electrically-distinct conductive paths;

a first electrical insulator sized smaller than said insulating base strip and overlaying a substantial portion of said conductive layer, said first insulator having at least a first cutout portion and a second cutout portion spaced from said first base end, said first cutout portion exposing a limited area of a first of said at least three conductive paths and said second cutout portion exposing a limited area of a second and a third of said at least three conductive paths;

at least two electrode materials wherein a first material of said at least two electrode materials is a reagent for measuring the concentration of said at least one analyte and wherein a second material of said at least two electrode materials is a material suitable for use as a reference material and for measuring the resistance of said fluid sample, said first material being disposed in said first cutout portion and said second material being disposed in said second cutout portion, said second material being scored to isolate said second material disposed on said second of said at least three conductive paths from said second material disposed on said third of said at least three conductive paths;

a second electrical insulator sized to fit over and coextensive with said first electrical insulator, said second insulator having an opening configured to expose an area of said first insulator a limited distance from said first base end of said insulating base strip, said area including said at least two cutout portions of said first insulator; and

a third electrical insulator sized to fit over and coextensive with said second insulator creating a sample fluid channel, said third insulator having a

third insulator vent aperture spaced from said first base end and configured to expose at least a small portion of said opening of said second insulator.

31. The strip of Claim 30 wherein said sample fluid channel has a volume of 1.44 microliters.

32. The strip of Claim 30 wherein said sample fluid channel is hydrophilic.

33. The device of Claim 30 wherein said first material and said second material are mixtures having starting components comprising a redox mediator, a stabilizer, a binder, a surfactant, and a buffer.

34. The strip of Claim 33 wherein said redox mediator is at least one metal complex selected from the group consisting of ferrocene, ferrocene derivatives and potassium ferricyanide, said stabilizer is a polyalkylene glycol, said binder is a cellulose material, said surfactant is a polyoxyethylene ether, and said buffer has a pH of about 5 to about 6.

35. The strip of Claim 34 wherein said mediator is potassium ferricyanide, said stabilizer is polyethylene glycol, said binder is methyl cellulose, said surfactant is t-octylphenoxypolyethoxyethanol, and said buffer is a citrate buffer.

36. The strip of Claim 35 wherein said first reagent is made of a mixture having starting components comprising about 1wt% of said potassium ferricyanide, about 2.5wt% of said polyethylene glycol, about 1wt% of said methyl cellulose, and about 0.03wt% of said t-octylphenoxypolyethoxyethanol in said citrate buffer.

37. The strip of Claim 35 wherein said second reagent is made of a mixture having starting components comprising about 6.5wt% of said potassium ferricyanide, about 2.5wt% of said polyethylene glycol, about 1wt% of said methyl cellulose, about 0.03wt% of said t-octylphenoxypolyethoxyethanol, and about 1wt% of an enzyme in said citrate buffer.

38. The strip of Claim 37 wherein said enzyme is glucose oxidase.

39. The strip of Claim 30 wherein said insulating base strip, said first electrical insulator, said second electrical insulator, and said third electrical insulator are made from a plastic material selected from the group consisting of polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic, and polystyrene.

40. A method of making multiple, disposable sensors wherein each sensor has a first working electrode, a second working electrode and a reference electrode, wherein said first working electrode contains an enzyme capable of catalyzing a reaction involving a substrate for the enzyme, said first working electrode, said second working electrode and said reference electrode being disposed in a fluid sample channel for measuring a fluid sample, said method comprising:

obtaining a base strip of an insulating material having a layer of conductive material disposed thereon, said base strip having a first edge and a second edge;

scribing in said conductive material a plurality of lines in a repetitive pattern wherein said plurality of lines contain a repetitive pattern forming three conductive paths in each of said repetitive pattern;

disposing a first middle layer of insulating material over said base strip, said first middle layer having a repetitive pattern of three cutouts wherein each cutout of each of said repetitive pattern exposes an electrode

portion of each of said three conductive paths of each repetitive pattern wherein said repetitive pattern of said three cutouts are spaced from said first edge of said base strip, and wherein said first middle layer is sized to expose a contact portion of each of said three conductive paths of each repetitive pattern for a distance from said second edge of said base strip;

disposing a first reagent material on two of said three cutouts of each repetitive pattern and a second reagent material on the other of said three cutouts of each repetitive pattern;

drying said first reagent material and said second reagent material;

overlaying a second middle layer of insulating material over and coextensive with said first middle layer; said second middle layer having a plurality of elongated cutout portions in a repetitive pattern wherein each of said elongated cutout portions exposes a corresponding repetitive pattern of said three cutouts said first middle layer;

disposing a top layer of insulating material over and coextensive with said second middle layer, said top layer having a plurality of vent openings in a repetitive pattern wherein each of said vent openings exposes a portion of a corresponding repetitive pattern of said elongated cutout portion furthest from said first edge of said base strip; and

separating each of said repetitive pattern forming one of each of said disposable sensors.

41. The method of Claim 40 further comprising drying said first reagent material and said second reagent material at a temperature and for a length of time sufficient to allow said first reagent material and said second reagent material to solidify and adhere to each of said electrode portion of each of said repetitive pattern of said three conductive paths.

42. The method of Claim 40 further comprising cutting along said first edge of each of said sensors and transverse to said sensors a predetermined distance creating a sample inlet port.

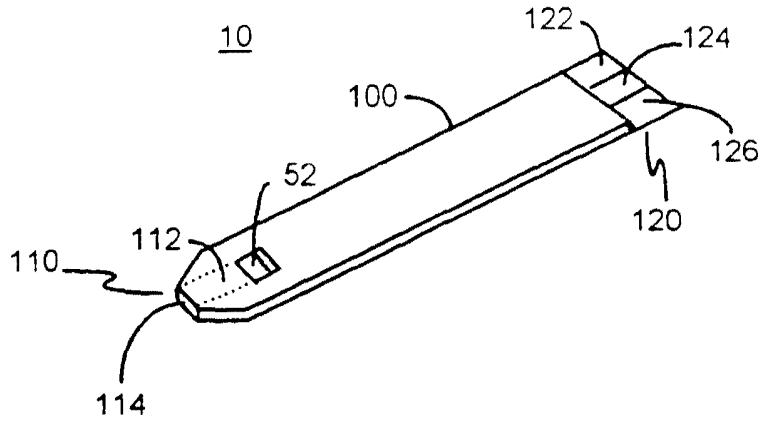

10
122
124
100
126
52
120
112
110
114

Fig. 1

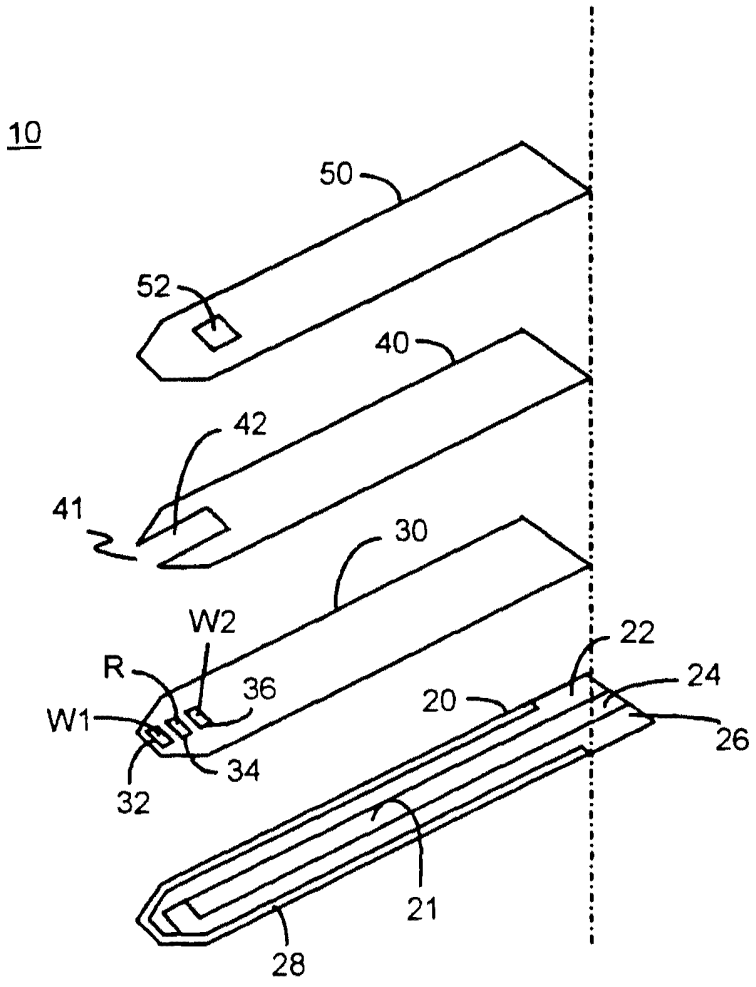

10
50
52
40
42
41
30
W2
22
R
24
36
20
W1
26
34
32
21
28

Fig. 2

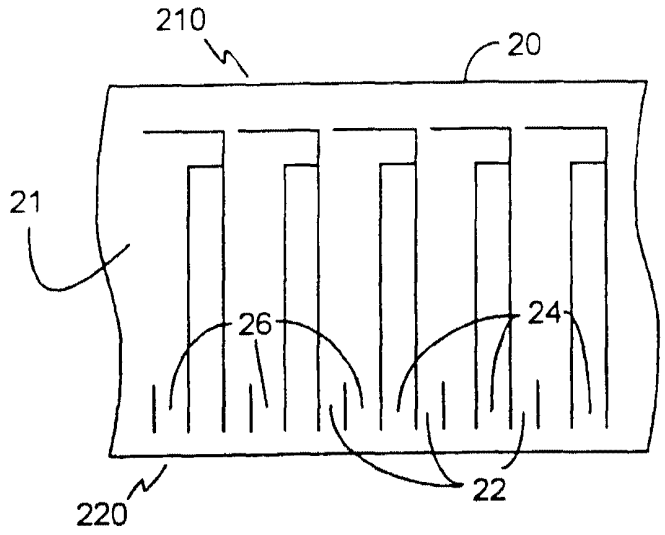


Fig. 3 A

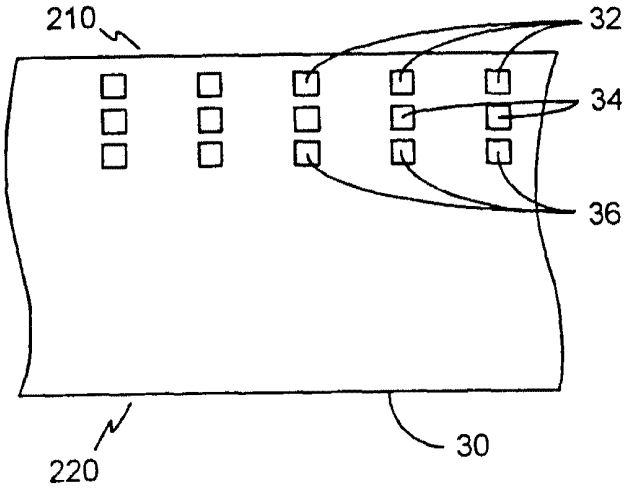


Fig. 3 B

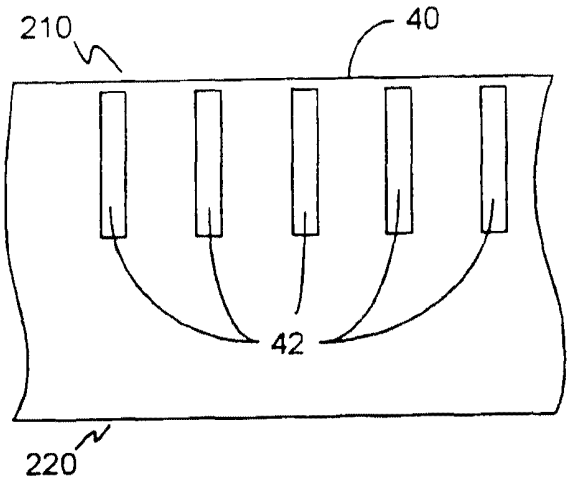


Fig. 3 C

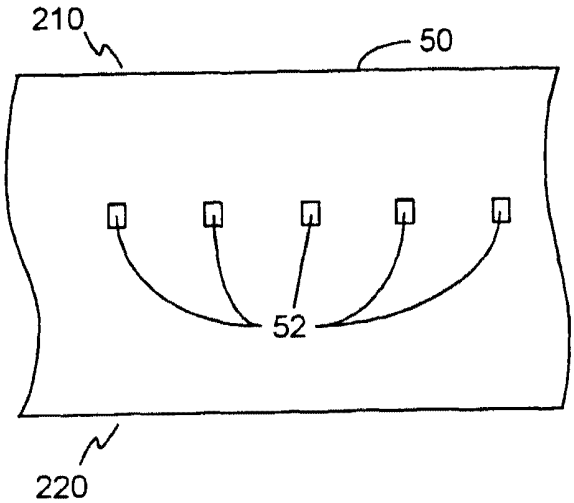


Fig. 3 D

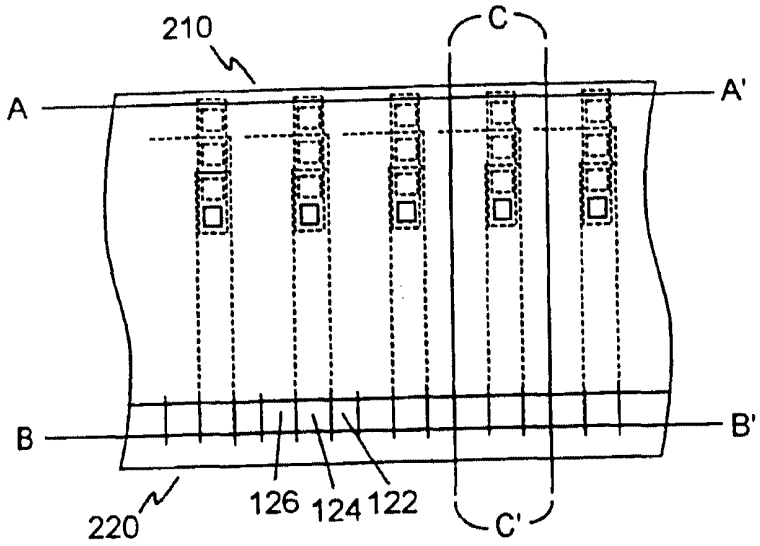


Fig. 3 E

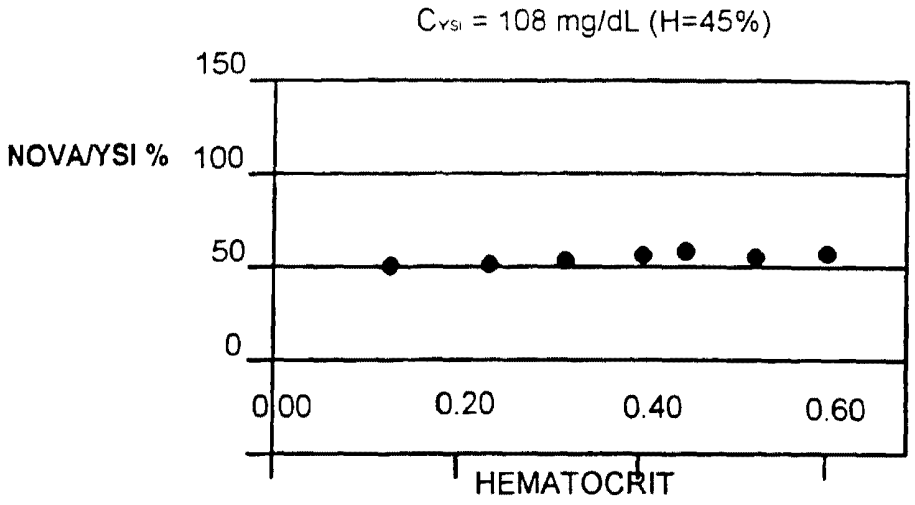

C_{YSI} = 108 mg/dL (H=45%)
NOVA/YSI %
150
100
50
0
0.00
0.20
0.40
0.60
HEMATOCRIT

Fig. 4A

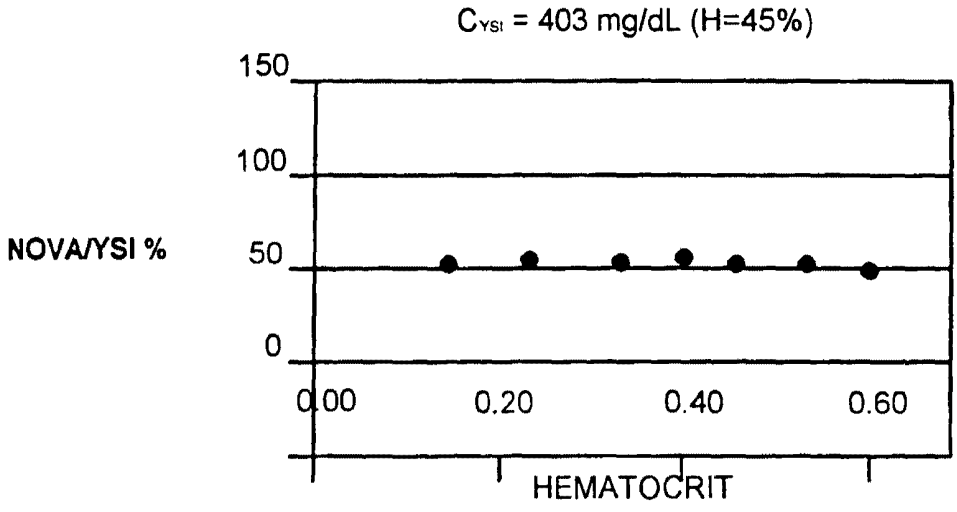

C_{YSI} = 403 mg/dL (H=45%)
NOVA/YSI %
150
100
50
0
0.00
0.20
0.40
0.60
HEMATOCRIT

Fig. 4B

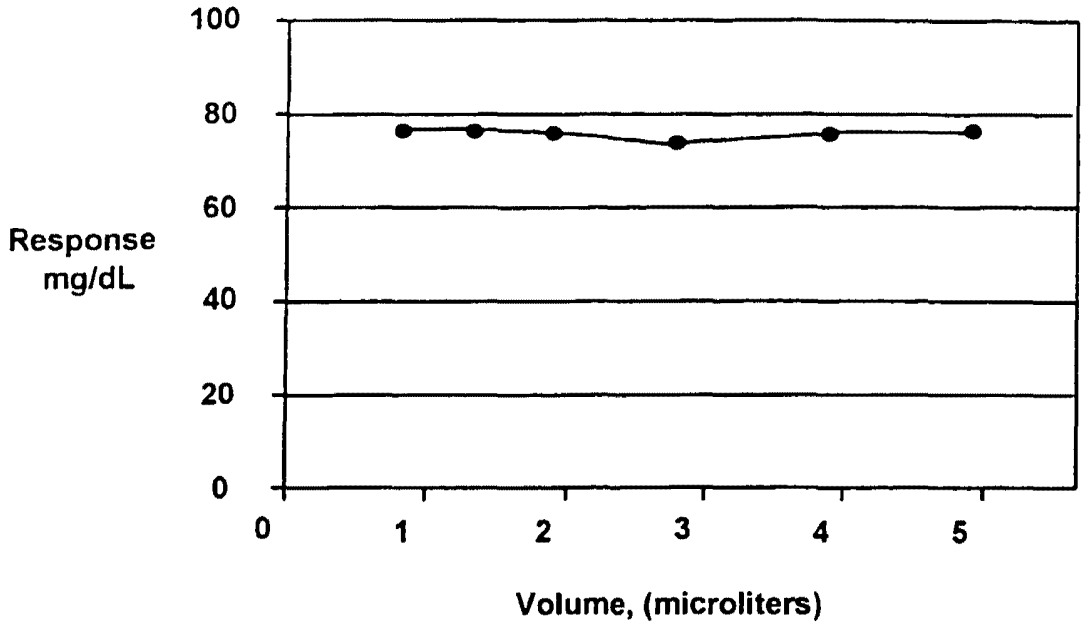

100
80
60
40
20
0
Response
mg/dL
0
1
2
3
4
5
Volume, (microliters)

Fig. 5

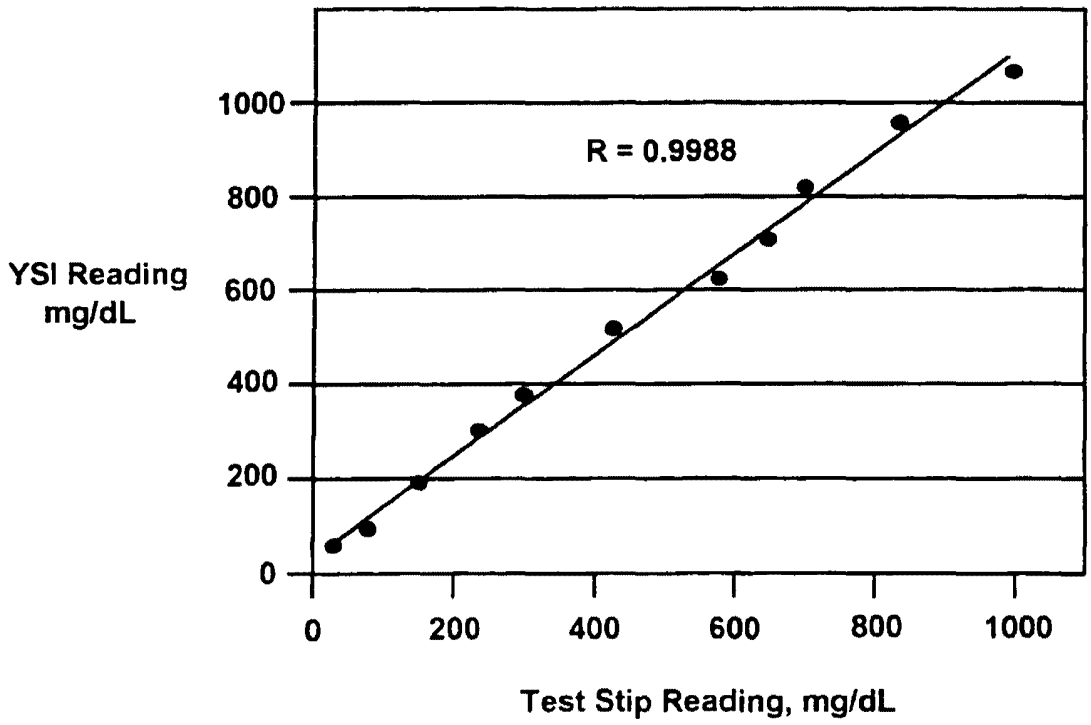

1000
800
600
400
200
0
YSI Reading
mg/dL
R = 0.9988
0
200
400
600
800
1000
Test Stip Reading, mg/dL

Fig. 6

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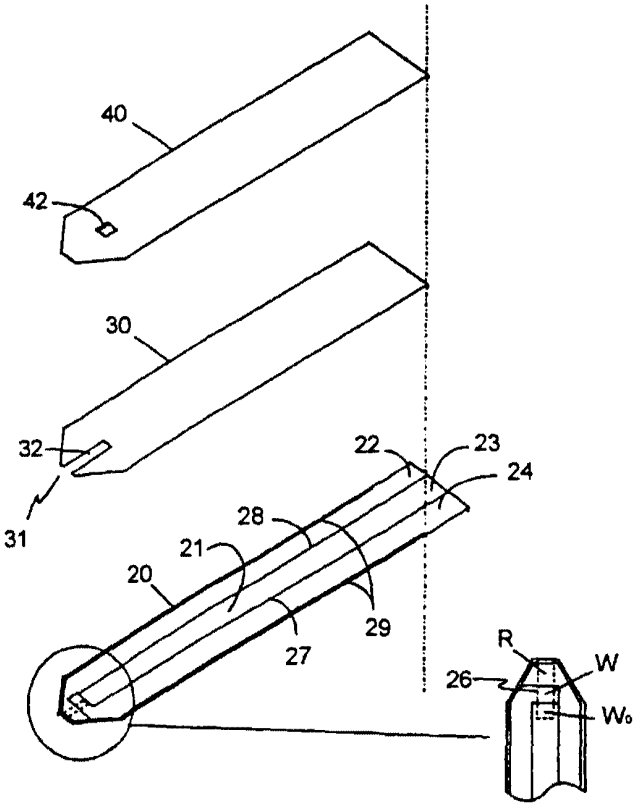
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[Continued on next page]

(54) Title: DISPOSABLE SUB-MICROLITER VOLUME SENSOR AND METHOD OF MAKING

(57) Abstract: A disposable electrode strip for testing a fluid sample including a laminated strip with a first and second end, a vent, an open path for receiving a fluid sample of less than one microliter beginning from the first end and connecting to the vent, a working electrode, a reference electrode and a pseudo-working electrode embedded in the laminated strip within the open path and proximate to the first end, a reagent matrix coextensive within the open path and covering the three electrodes, and conductive contacts located at the second end of the laminated strip.



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DISPOSABLE SUB-MICROLITER VOLUME SENSOR AND METHOD OF MAKING

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to electrochemical sensors that can be used for the quantification of a specific component or analyte in a liquid sample. Particularly, this invention relates to a new and improved electrochemical sensor and to a new and improved method of fabricating electrochemical sensors. More particularly, this invention relates to a disposable electrochemical sensor that is inexpensive to manufacture. Even more particularly, this invention relates to a disposable electrochemical sensor that gives accurate readings and requires only about 0.2 microliter of fluid sample. Still even more particularly, this invention relates to disposable electrochemical sensors which are used for performing electrochemical assays for the accurate determination of analytes in physiological fluids.

2. Description of the Prior Art

Biosensors have been used in the determination of concentrations of various analytes in fluids for more than three decades. Of particular interest is the measurement of blood glucose. It is well known that the concentration of blood glucose is extremely important for maintaining homeostasis. Products that measure fluctuations in a person's blood sugar, or glucose levels, have become everyday necessities for many of the nation's millions of diabetics. Because this disorder can cause dangerous anomalies in blood chemistry and is believed to be a contributor to vision loss and kidney failure, most diabetics need to test themselves periodically and adjust their glucose level accordingly, usually with insulin injections. If the concentration of blood glucose is below the normal range, patients can suffer from unconsciousness and lowered blood pressure which may even result in death. If the blood glucose concentration is higher than the normal range, the excess blood glucose can result in synthesis of fatty acids

and cholesterol, and in diabetics, coma. Thus, the measurement of blood glucose levels has become a daily necessity for diabetic individuals who control their level of blood glucose by insulin therapy.

Patients who are insulin dependent are instructed by doctors to check their blood-sugar levels as often as four times a day. To accommodate a normal life style to the need of frequent monitoring of glucose levels, home blood glucose testing was made available with the development of reagent strips for whole blood testing.

One type of blood glucose biosensors is an enzyme electrode combined with a mediator compound which shuttles electrons between the enzyme and the electrode resulting in a measurable current signal when glucose is present. The most commonly used mediators are potassium ferricyanide, ferrocene and its derivatives, as well as other metal-complexes. Many sensors based on this second type of electrode have been disclosed. Examples of this type of device are disclosed in the following patents.

U.S. Patent No. 5,628,890 (1997, Carter et al.) discloses an electrode strip having an electrode support, a reference or counter electrode disposed on the support, a working electrode spaced from the reference or counter electrode on the support, a covering layer defining an enclosed space over the reference and working electrodes and having an aperture for receiving a sample into the enclosed space, and a plurality of mesh layers interposed in the enclosed space between the covering layer and the support. The covering layer has a sample application aperture spaced from the electrodes. The working electrode includes an enzyme capable of catalyzing a reaction involving a substrate for the enzyme and a mediator capable of transferring electrons between the enzyme-catalyzed reaction and the working electrode.

This device proposes to reduce the effect of hematocrit on the sensor readings. According to the disclosure, this results from the downstream spacing of the reference electrode relative to the working electrode in combination with the thin layer of the sample solution created by the mesh layers.

U.S. Patent No. 5,708,247 (1998, McAleer et al.) discloses a disposable glucose test strip having a substrate, a reference electrode, a working electrode,

and a means for making an electrical connection. The working electrode has a conductive base layer and a coating layer disposed over the conductive base layer. The coating layer is a filler having both hydrophobic and hydrophilic surface regions which form a network, an enzyme and a mediator.

U.S. Patent No. 5,682,884 (1997, Hill et al.) discloses a strip electrode with screen printing. The strip has an elongated support which includes a first and second conductor each extending along the support. An active electrode, positioned to contact the liquid mixture and the first conductor, has a deposit of an enzyme capable of catalyzing a reaction and an electron mediator. A reference electrode is positioned to contact the mixture and the second conductor.

U.S. Patent No. 5,759,364 (1998, Charlton et al.) discloses an electrochemical biosensor having an insulating base plate bearing an electrode on its surface which reacts with an analyte to produce mobile electrons. The base plate is mated with a lid of deformable material which has a concave area surrounded by a flat surface so that when mated to the base plate there is formed a capillary space into which a fluid test sample can be drawn. The side of the lid facing the base is coated with a polymeric material which serves to bond the lid to the base plate and to increase the hydrophilic nature of the capillary space.

U.S. Patent No. 5,762,770 (1998, Pritchard et al.) discloses an electrochemical biosensor test strip that has a minimum volume blood sample requirement of about 9 microliters. The test strip has a working and counter electrodes that are substantially the same size and made of the same electrically conducting material placed on a first insulating substrate. Overlaying the electrodes is a second insulating substrate which includes a cutout portion that forms a reagent well. The cutout portion exposes a smaller area of the counter electrode than the working electrode. A reagent for analysis of an analyte substantially covers the exposed areas of the working and counter electrodes in the reagent well. Overlaying the reagent well and affixed to the second insulating substrate is a spreading mesh that is impregnated with a surfactant.

U.S. Patent No. 5,755,953 (1998, Henning et al.) discloses a reduced-interference biosensor. The device generally comprises an electrode used to

electrochemically measure the concentration of an analyte of interest in a solution. The device includes a peroxidase enzyme covalently bound to microparticle carbon and retained in a matrix in intimate contact with the electrode. According to this disclosure, it is the enzyme/microparticle carbon of the device which provides a composition which is displays little sensitivity to known interfering substances.

U.S. Patent No. 5,120,420 (1992, Nankai et al.) discloses a biosensor with a base board having an electrode system mainly made of carbon, an insulating layer, a reaction layer containing an enzyme layer thereon, a spacer and a cover. The spacer creates a channel with an inlet and an outlet for holding a sample.

PCT Patent Application No. WO 98/35225 (1998, Heller et al.) discloses a sensor designed to determine the amount and concentration of an analyte in a sample having a volume of less than about one microliter. The sensor has facing working and reference electrodes with an optional sorbent spacer. The working electrode is coated with a reagent layer containing a non-leachable redox mediator and an enzyme. This device, which is capable of using a test sample volume of less than 1 microliter, requires the use of a sorbent material within the sample chamber in order to reduce the volume requirement and to introduce a hydrophilic character to the chamber in order for the sample to flow into such chamber.

However, most of the remaining prior art devices require a test sample volume of greater than 2 microliters. This volume of test sample can only be obtained from a patient, for example, using a needle and syringe, or by lancing a portion of the skin such as the fingertip and "milking" the area to obtain a useful sample volume. These procedures are inconvenient for the patient, and often painful, particularly when frequent samples are required. Less painful methods for obtaining a sample are known such as lancing the arm or thigh, which have a lower nerve ending density. However, lancing the body in the arm or thigh typically produces submicroliter sample volumes of blood because these areas are not heavily supplied with near-surface capillary blood vessels. Because the present invention requires as little as 0.2 microliters of blood, patients who must

make several blood glucose measurements a day may obtain blood samples from these preferred areas.

Additional shortcomings of the prior art devices are that they have a more limited linear range, usually up to about 600 mg/dL. Further, they require a relatively longer waiting time for development of a steady-state response before a reading can be achieved.

Because of the importance of obtaining accurate glucose readings, it would be highly desirable to develop a reliable and user-friendly electrochemical sensor which does not have all of the drawbacks mentioned above. Therefore what is needed is an electrochemical sensor which requires less sample volume than previously required by the prior art. What is further needed is an electrochemical sensor which has a wide linear measurement range; that is, a sensor useable over a wider glucose concentration. What is still further needed is an electrochemical sensor which has a relatively short wait time for development of a steady-state response.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an improved electrochemical sensor which combines an enzyme and a mediator. It is a further object of the present invention to provide an electrochemical sensor which requires less sample volume than previously required by the prior art. It is still another object of the present invention to provide an electrochemical sensor which can measure a small volume of sample without the use of a mesh layer in the sample path. It is yet another object of the present invention to provide an electrochemical sensor which has a wide linear measurement range and a relatively short wait time for development of a steady-state response.

The present invention achieves these and other objectives by providing an electrochemical sensor which requires a sample size of only about 0.2 microliters and does not use a mesh layer in the sample path as a means of achieving a reduced size of the sample. Further the present invention uses a reagent composition which allows readings, which correlate very closely to the analyte concentration in the fluid sample, to be taken 20 seconds after the fluid sample

enters the sample channel. The present invention has a laminated, elongated body having a sample fluid channel connected between an opening on one end of the laminated body and a vent hole spaced from the opening. The sample fluid channel is sized to optimize the quick flow of a sample such as whole blood into the channel. The rapid uptake of the sample allows the electrode reactions to reach steady-state faster, thus resulting in obtaining an analyte reading more quickly. Within the fluid channel lies at least one working electrode and a reference electrode, preferably a working electrode, a reference electrode and a pseudo-working electrode. The arrangement of the working electrode and the reference electrode is not important for purposes of the results obtained from the electrochemical sensor. The working electrode, the reference electrode and the pseudo-working electrode are each in electrical contact with separate conductive conduits, respectively. The separate conductive conduits terminate and are exposed for making an electrical connection to a reading device on the end opposite the open channel end of the laminated body.

The laminated body has a base insulating layer made from a plastic material. The base insulating layer has a conductive layer on one side. The conductive layer may be deposited on the insulating layer by screen printing, by vapor deposition, or by any method that provides for a conductive layer which adheres to the base insulating layer and substantially covers all of the base insulating layer. The vapor-deposited conductive layer is separated into conductive conduits by etching/scribing the conductive layer. The etching process may be accomplished chemically, by mechanically scribing lines in the conductive layer, by using a laser to scribe the conductive layer into separate conductive conduits, or by any means that will cause a break between and among the separate conductive conduits required by the present invention. The preferred conductive coatings are gold film or a tin oxide/gold film composition/layer.

It should be pointed out that the gold film or tin oxide/gold film itself cannot function as a reference electrode. To make the reference electrode work, there must be a redox reaction (e.g., $Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$) at the electrically

conducting material when a potential is applied. Therefore, a redox mediator must be present at the conducting material used for the reference electrode.

The unique feature of the present invention is its ability to measure sample sizes as small as 0.15 microliters without using opposing working and reference electrodes and a sorbent/mesh layer therebetween to reduce the required sample volume for measurement. This is possible because of the combination of material used for the base insulating layer with conductive coating, and the unique method of forming the conductive conduits thereon.

The laminated body also has a middle insulating layer on top of the base layer. The middle layer is also made of a plastic insulating material and creates the sample fluid channel of the laminated body. It contains a U-shaped cutout on one end which overlays the electrode portion of the conductive conduits on the base layer with the open end corresponding to the open end of the laminated body described earlier.

The thickness of the middle layer must be of sufficient thickness for loading a sufficient amount of chemical reagent for use as an electrochemical sensor while maintaining a flow-channel dimension having optimum blood flow characteristics. The U-shaped cutout contains chemical reagent. The chemical reagent has a redox mediator with at least one of a stabilizer, a binder, a surfactant, a buffer, and an enzyme capable of catalyzing a reaction involving a substrate for the enzyme. The redox mediator is capable of transferring electrons between the enzyme-catalyzed reaction and the working electrode. It also makes the reference electrode function.

The laminated body of the present invention has a top layer with a vent opening. The vent opening is located such that at least a portion of the vent opening overlays the bottom of the U-shaped cutout exposing a portion of the chemical reagent of the middle insulating layer. The vent allows air within the sample fluid channel to escape as the sample fluid enters the open end of the laminated body. The sample fluid generally fills the sample fluid channel by capillary action. In small volume situations, the extent of capillary action is dependent on the hydrophobic/hydrophilic nature of the surfaces in contact with the fluid undergoing capillary action. This is also known as the wetability of the

material. Capillary forces are enhanced by either using a hydrophilic insulating material to form the top layer, or by coating at least a portion of one side of a hydrophobic insulating material with a hydrophilic substance in the area of the top layer that faces the sample fluid channel between the open end of the laminated body and the vent opening of the top layer. It should be understood that an entire side of the top layer may be coated with the hydrophilic substance and then bonded to the second middle layer.

The three layers of the laminated body may be made from any dielectric material. The preferred material is a plastic material. Examples of acceptable compositions for use as the dielectric material are polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic, and polystyrene.

The electrode portions, located within the sample fluid channel, contain reagent material for the working electrode (W), the reference electrode (R) and the pseudo-working electrode (W_0). A reagent mix is disposed into the fluid channel thus covering the electrode portions of the base insulating layer and the conductive conduits. A sufficient amount of reagent mix is deposited within the U-shaped cutout of the middle insulating layer to substantially cover all of the conductive surface delineated by the U-shaped cutout. The amount of the reagent mix used is such that the reagent matrix created upon drying is sufficient for use as an electrochemical sensor yet provides enough empty space above the reagent matrix to allow rapid blood flow through the fluid channel. The reagent matrix has a redox mediator with at least one of a stabilizer, a binder, a surfactant, a buffer, and an enzyme capable of catalyzing a reaction involving a substrate for the enzyme.

The possible electrode arrangements within the sample fluid channel may be W- R-W_0, W-W_0-R, R-W-W_0, R-W_0-W, W_0-W-R or W_0-R-W with the arrangement listed as the arrangement of electrodes would appear from the open end of the laminated body to the vent opening. The preferred position was found to be R-W-W_0; that is, as the sample fluid entered the open end of the laminated body, the fluid would cover R first, then W, then W_0.

The pseudo-working electrode, W_0, is positioned so that the sample fluid reaches it last. The resulting current at W_0 thus triggers the reading meter to start the measurement and analyte concentration determination process. Such an arrangement obviates reliability and accuracy problems due to an insufficient sample fluid size. It should be pointed out that W_0 can also be used as a counter electrode. The resulting three-electrode system (i.e. working electrode, reference electrode and counter electrode) would be used in the case of a sample fluid having a large IR drop. It should also be pointed out that W_0, combined with R, can be used to measure the resistance of the sample fluid. The resulting resistance could be used to estimate the hematocrit of a blood sample and therefore to correct the hematocrit interference.

All of the advantages of the present invention will be made clearer upon review of the detailed description, drawings and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a perspective view of the present invention showing the open end, the vent and the electrical contact points of the laminated body.

FIGURE 2 is an exploded, perspective view of the present invention showing the various layers of the laminated body.

FIGURES 3 is a cross-sectional view of the present invention of Fig. 1

FIGURES 4A, 4B and 4C are top views of a segment of a strip of each layer of the present invention showing the patterns for making multiple sensors of the present invention.

FIGURE 4D is a top view of a segment of the laminated strip of the present invention showing the patterns for making multiple sensors of the present invention.

FIGURE 5 is a correlation of sample volume on the concentration response of the present invention.

FIGURE 6 is a correlation curve of the concentration readings using sensors of the present invention versus the concentration readings of obtained on the same samples using a YSI glucose analyzer.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The preferred embodiment of the present invention is illustrated in FIGURES 1-6 . Figure 1 shows a sensor **10** of the present invention. Sensor **10** has a laminated body **100**, a fluid sampling end **110**, an electrical contact end **120**, and a vent opening **42**. Fluid sampling end **110** includes a sample fluid channel **112** between a sampling end aperture **114** and vent opening **42**. Electrical contact end **120** has three discreet conductive contacts **122**, **123** and **124**.

Referring now to Figure 2, laminated body **100** is composed of a base insulating layer **20**, a middle layer **30**, and a top layer **40**. All layers are made of a dielectric material, preferably plastic. Examples of a preferred dielectric material are polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic and polystyrene. Base insulating layer **20** has a conductive layer **21** on which is delineated a first conductive conduit **22**, a second conductive conduit **23** and a third conductive conduit **24**. Conductive conduits **22**, **23** and **24** may be formed by scribing or scoring the conductive layer **21** as illustrated in Fig. 2 and shown as scribe line **27** and **28** or by silk-screening the conductive conduits **22**, **23** and **24** onto base layer **20**. Scribing or scoring of conductive layer **21** may be done by mechanically scribing the conductive layer **21** sufficiently to create the three independent conductive conduits **22**, **23** and **24**. The preferred scribing or scoring method of the present invention is done by using a carbon dioxide (CO_2) laser, a YAG laser or an eximer laser. An additional scoring line **29** (enlarged and not to scale; for illustrative purposes only) may be

made. but is not necessary to the functionality of sensor **10**, along the outer edge of base layer **20** in order to avoid potential static problems which could give rise to a noisy signal. Conductive layer **21** may be made of any electrically conductive material, preferably gold or tin oxide/gold. A useable material for base layer **20** is a tin oxide/gold polyester film (Cat. No. FM-1) or a gold polyester film (Cat. No. FM-2) sold by Courtaulds Performance Films, Canoga Park, California.

Middle layer **30** has a U-shaped channel cutout **32** located at middle layer sensor end **31**. The length of channel cutout **32** is such that when middle layer **30** is layered on top of base layer **20**, electrode areas **W**, **R** and $\mathbf{W_0}$ are within the space defined by channel cutout **32**. The thickness of middle layer **30** was found to be critical for the speed of the sample fluid flow into sample fluid channel **112,** which is filled by capillary action of the sample fluid. Channel cutout **32** holds the reagent matrix **50**, more clearly shown in Fig. 3, forming the working electrode, the reference electrode and the pseudo-working electrode. Typically, the reagent matrix **50** must be loaded with a redox mediator to make the reference electrode function. If **R** is not loaded with a redox reagent or mediator, working electrode **W** and $\mathbf{W_0}$ will not work. Electrode areas **W**, $\mathbf{W_0}$ and **R** are loaded preferably with the same chemical reagent. The reagents preferably contain an oxidized form of a redox mediator, a stabilizer, a binder, a surfactant, a buffer, and an enzyme. Typically, the redox mediator may be at least one of ferrocene, potassium ferricyanide, other ferrocene derivatives, or other organic and inorganic redox mediators. The preferred stabilizer is polyethylene glycol, the preferred binder is methyl cellulose, the preferred surfactant is t-octylphenoxypolyethoxyethanol, and the preferred buffer is a citrate buffer. The enzyme is capable of catalyzing a reaction involving a substrate for the enzyme or a substrate catalytically reactive with an enzyme and a mediator capable of transferring electrons transferred between the enzyme-catalyzed reaction and the working electrode to create a current representative of the activity of the enzyme or substrate and representative of the compound. An example of such an enzyme is glucose oxidase.

Top layer **40**, which is placed over and coextensive with middle layer **30**, has a vent opening **42** spaced from fluid sample end **110** of sensor **10** to insure that sample fluid in fluid channel **112** will completely cover electrode areas **W**, **R** and $\mathbf{W_0}$. Vent opening **42** is placed in top layer **40** so that it will align somewhat with the bottom of channel cutout **32** of middle layer **30**, the bottom meaning the channel cutout **32** located furthest from sensor end **31**. Preferably, vent opening **42** will expose a portion of and partially overlay the bottom of the U-shaped cutout **32** of middle layer **30**. Figure 3 shows an enlarged cross-sectional view of the various layers of the present invention. The layers are not to scale in order that the relationship of each component of the present invention may be better understood by those skilled in the art, especially scribe lines **27** and **28**.

Preparation of Electrode Reagent Matrix

The electrode reagent matrix comprises the oxidized form of a redox mediator, a stabilizer, a binder, a surfactant, a buffer, and an enzyme. The oxidized form of the redox mediator, potassium ferricyanide, was found to be stable in the matrix. Suitable potassium ferricyanide is available from Sigma Chemical, St. Louis, MO (Cat. No P3667). The quantity used in the formulation must be sufficient to attain a workable linear range. The enzyme must also have sufficient activity, purity and stability. A commercially available glucose oxidase may be obtained from Biozyme, San Diego, California as Cat. No. G03A, about 270U/mg. The stabilizer must be sufficiently water-soluble and be capable of stabilizing both the mediator and the enzyme. The preferred stabilizer is polyethylene glycol (Cat. No. P4338, Sigma Chemicals, St. Louis, MO). The binder should be capable of binding all other chemicals in the reagent matrix in electrode areas **W**, **R** and $\mathbf{W_0}$ to the conductive surface/layer **21** of base layer **20**. The preferred binder is Methocel 60 HG (Cat. No. 64655, Fluka Chemical, Milwaukee, WI). The buffer solution must have sufficient buffer capacity and pH value to optimize the enzyme reaction. A 0.05M citrate buffer is preferred. Citric acid and sodium citrate used in making the citrate buffer may be obtained from Sigma Chemical. The surfactant is necessary to facilitate dispensing of the

electrode reaction matrix into channel cutout **32** as well as for quickly dissolving the dry chemical reagents involved in forming the reagent matrix. The amount and type of surfactant is selected to assure the previously mentioned functions and to avoid a denaturing effect on the enzyme. The preferred surfactant is Triton X-100 available from Fluka Chemical, Milwaukee, WI (Cat. No. 94443). The reagent matrix is obtained by preparing a reagent mix as follows:

Step 1: Prepare 50 mM citrate buffer (pH 5.7) by dissolving 0.1512 grams citric acid and 1.2580 grams sodium citrate in 100 ml of deionized water.

Step 2: Prepare a 1% methocel 60HG solution by stirring 1 gram of methocel in 100 ml of citrate buffer from Step 1 for 12 hours.

Step 3: Add 0.3 ml of 10% Triton X-100 into the methocel solution.

Step 4: Add 2.5 grams of polyethylene glycol into the solution from Step 3.

Step 5: While stirring, add 6.5 grams potassium ferricyanide to the solution of Step 4.

Step 6: Add 1.0 gram of glucose oxidase to the solution of Step 5 and stir for 10 minutes or until all solid materials are completely dissolved.

Electrode Construction

A piece of a gold or tin oxide/gold polyester film available from Courtaulds Performance Films is cut to shape, as illustrated in Fig. 2, forming base layer **20** of sensor **10**. A CO_2 laser is used to score the gold or tin oxide/gold polyester film (25W laser available from Synrad, Inc., San Diego, CA). As illustrated in Fig. 2, the film is scored by the laser creating scoring line **27** and **28** such that two electrodes at sample fluid end **110** and three contact points **122**, **123** and **124** were formed at electrical contact end **120**. The scoring line is very thin but sufficient to create two separate electrical conductors. An additional scoring line **29** made be made, but is not necessary, along the outer edge of base layer **20** to avoid potential static problems which could cause a noisy signal from the finished sensor **10**.

A piece of double-sided tape (Arcare® 7840) available from Adhesive Research, Glen Rock. PA, is cut to size and shape forming middle layer **30** with U-shaped channel **32** so that it will cover a majority of the conductive layer **21** of base layer **20** except for exposing a small electrical contact area at electrical contact end **120** illustrated in Fig. 1. The U-shaped channel **32** is cut by using the CO_2 laser. Middle layer **30** is then layered onto base layer **20**. As mentioned earlier, this middle layer **30** serves as a spacer and defines the size of the fluid sample channel **112**. It also defines the electrode area **26** which holds the electrode reagent matrix **50**. Its width and length is optimized to provide for a relatively quick moving fluid sample. The preferred size of U-shaped channel **32** is about 0.039 in. (1.0 mm) wide by about 0.134 in. (3.4 mm) long.

1.0 microliters of reagent mix is dispensed into channel **32** to form electrodes **W**, **R** and $\mathbf{W_0}$. The reagent mix is a mixture of a redox mediator, a stabilizer, a binder, a surfactant, a buffer, and an enzyme. The preferred composition for the reagent mix is made by mixing the following percentages (W/W%) of the following ingredients: about 6.5% potassium ferricyanide, about 2.5% polyethylene glycol, about 1% methocel 60 HG, about 0.03% Triton X-100, about 0.05M citrate buffer (pH 5.7), and about 1% glucose oxidase. After the addition of the reagent mix, the device was dried in an oven at 55 °C for about 2 minutes.

After drying, a piece of a transparency film (Cat. No. PP2200 or PP2500 available from 3M) is fashioned into top layer **40**. A rectangular vent hole **42** is made using the CO_2 laser previously mentioned. The preferred size of vent hole **42** is about 0.039 in. (1.0 mm) by about 0.051 in. (1.30 mm). Vent hole **42** is located approximately 0.087 in. (2.2 mm) from fluid end **110** of sensor **10**. Top layer **40** is aligned and layered onto middle layer **30** to complete the assembly, as illustrated in Fig. 1, of sensor **10**.

Although the description of electrode construction above describes construction for a single sensor, the design and materials used are ideal for making multiple sensors from one piece of each layer material as shown in Figs. 4A-4C. This is accomplished by starting with a relative large piece of base layer

20 having conducting layer **21** thereon. A plurality of scored lines **27** and **28** are made into conductive layer **21** such that a repetitive pattern, as illustrated in Fig. 4A, is created using the preferred scribing method described previously whereby each pattern will eventually define the three conductive paths **22**, **23** and **24** for each sensor. Similarly, a large piece of middle layer **30** having a plurality of elongated cutouts **32** in a repetitive pattern and illustrated in Fig. 4B is layered onto base layer **20**. The large piece of middle layer **30** is sized to fit over base layer **20** in such that the plurality of elongated cutouts **32** are aligned over the areas where the scribe lines **27** and **28** intersect exposing three distinct electrode areas **W**, **R** and $\mathbf{W_0}$, and exposing the plurality of conductive contacts **122**, **123** and **124** located at the opposite edge of the strip. The size of each cutout and the amount of reagent mix disposed in each channel **32** are similar to that disclosed above. After dispensing the reagent mix into the respective cutouts, the reagent mix is dried such that each elongated cutout **32** of middle layer **30** contains a thin layer of the reagent matrix. A top layer **40** comparably-sized to and coextensive with middle layer **30** having a plurality of vent openings **42** in a repetitive pattern, as shown in Fig. 4C, is layered onto middle layer **30**. Fig. 4D is a top view of the combined layers. The laminated strip created by the three layers **20**, **30** and **40** has a plurality of sensors **10** that can be cut from the laminated strip. The laminated strip is cut longitudinally along line A-A' at fluid sampling end **210** to form a plurality of sampling apertures **114** and longitudinally along line B-B' at electrical contact end **220** to form a plurality of conductive contacts **122**, **123** and **124**. The laminated strip is cut at predetermined intervals along lines C-C' forming a plurality of individual sensors **10**. Shaping of the fluid sampling end **120** of each sensor **10**, as illustrated in Fig. 1, may be performed if desired. It should be understood by those skilled in the art that the order in which the laminated strip can be cut is not important. For instance, the laminated strip may be cut at the predetermined intervals (C-C') and then the cuts along A-A' and B-B' can be made to complete the process.

The following examples illustrate the unique features of the present invention. All sensors of the present invention were tested on a breadboard glucose meter manufactured by Nova Biomedical Corporation of Waltham, MA. A potential of 0.35 Volts was applied across the working electrode and the reference electrode and the resultant current signals were converted to glucose concentrations. The readings were compared to readings (control readings) obtained on the same samples using a YSI Glucose Analyzer (Model 2300) available from Yellow Springs Instruments, Inc., Yellow Springs, OH.

Example 1

Demonstration of Minimum Sample Volumes Feature

The unique design of the present invention enables the measurement of sample sizes smaller than which have heretofore been possible. Blood samples are applied to the sensors and the samples travel along the fluid sample channel to the venting hole. The blood volume required for measurement of blood glucose is determined by the channel volume. The calculated volume for the present invention is 0.22 microliters. In order to test the volume effect on sensor response, different blood sample volumes were applied to the sensors and the resulting concentration readings were plotted against volume. The test data is shown in Fig. 5.

Sensors of the present invention show no dependence of the response on the sample volume down to a volume of less than 0.22 microliters. It was found that sensors of the present invention still gave reasonable readings on sample sizes as low as 0.15 microliters. This is possible because the hydrophilic character of the Reagent Matrix applied to W, R and W_0 permitted the sample to cover the electrode areas even though the blood volume did not fill the entire sample channel.

Example 2

Demonstration of Wide Linear Range and Precision Feature

A sample of venous blood was collected and separated into several aliquots. Each aliquot was spiked with different glucose concentrations ranging

from 35 to 1000 mg/dL. The aliquots were each measured with a YSI glucose analyzer and then with sensors of the present invention using the Nova glucose meter. Sensors of the present invention show a linear relationship of current response vs. glucose concentration from 35 to 1000 mg/dL. The concentration readings were plotted against the concentration values obtained using the YSI meter (the control) and are illustrated in Fig. 6.

A regression coefficient of 0.9976 indicated a near perfect match with the readings obtained with the YSI blood glucose analyzer. The same aliquots were tested using four different commercially-available sensors with their accompanying meters. The commercially-available sensors showed a linear response only up to about 600 mg/dL. Above the 500-600 mg/dL range, all commercially available sensors displayed "Hi" as the test result.

The precision of the sensors of the present invention was investigated at the same glucose level range from about 35 to 1000 mg/dL. Four different batches of sensors of the present invention were used in the precision tests. Typically, the relative standard deviation was about 5.0% and 3.6% for samples containing 100 and 300 mg/dL levels of glucose, respectively.

What is claimed is:

1. A disposable electrode strip for testing a fluid sample comprising:

a laminated strip having a first strip end, a second strip end and a vent opening spaced from said first strip end, said laminated strip comprising a base layer having a conductive layer disposed thereon and scribed to delineate three electrode paths, a channel forming layer carried on said base layer, and a cover;

an enclosed channel between said first strip end and said vent opening, said enclosed channel sized to hold a volume of said fluid sample less than one microliter;

a reagent matrix containing at least an enzyme and a redox mediator disposed on said base layer in said enclosed channel;

conductive contacts at said second strip end and insulated from said enclosed channel.

2. The electrode strip of Claim 1 wherein said enzyme is glucose oxidase.

3. The electrode strip of Claim 1 wherein said redox mediator is at least one metal complex.

4. The electrode strip of Claim 3 wherein said redox mediator is potassium ferricyanide and other inorganic and organic redox mediators.

5. The electrode strip of Claim 1 wherein said conductive coating is gold.

6. The electrode strip of Claim 1 wherein said conductive coating comprising gold and tin oxide.

7. The electrode strip of Claim 1 wherein said base layer, said channel forming layer and said cover are made of a plastic dielectric material.

8. The electrode strip of Claim 7 wherein said plastic material is selected from the group consisting of polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic, and polystyrene.

9. The electrode strip of Claim 1 wherein said enclosed channel is hydrophilic.

10. The electrode strip of Claim 1 wherein said enclosed channel has a volume of about 0.22 microliters.

11. The electrode strip of Claim 1 wherein said cover has a hydrophilic coating on at least one side.

12. The electrode strip of Claim 1 wherein said reagent matrix further contains at least one of a stabilizer, a binder, a surfactant, and a buffer.

13. The electrode strip of Claim 12 wherein said stabilizer is a polyalkylene glycol, said binder is a cellulose material, and said surfactant is a polyoxyethylene ether.

14. The electrode strip of Claim 13 wherein said stabilizer is polyethylene glycol, said binder is methyl cellulose, said surfactant is t-octylphenoxypolyethoxyethanol, and said buffer is a citrate buffer.

15. The electrode strip of Claim 14 wherein said reagent matrix is made from a mixture having starting components comprising about 1wt% to about 6.5wt% of said redox mediator, about 2.5wt% of said stabilizer, about 1wt% of said binder, about 0.03wt% of said surfactant, and about 1wt% of said enzyme in said citrate buffer.

16. The electrode strip of Claim 15 wherein said citrate buffer is about 0.05M.

17. The electrode strip of Claim 1 wherein said channel forming layer has a thickness sufficient to optimize the flow of said fluid sample along said open path.

18. The electrode strip of Claim 17 wherein said thickness is about 0.0035 inches (0.089 mm).

19. The electrode strip of Claim 15 wherein said potassium ferricyanide is 6.5wt%.

20. The electrode strip of Claim 15 wherein said enzyme is glucose oxidase.

21. The electrode strip of Claim 1 wherein said enclosed channel contains a working electrode, a pseudo-working electrode and a reference electrode.

22. The electrode strip of Claim 21 wherein said pseudo-working electrode is a counter electrode.

23. The electrode strip of Claim 21 wherein said pseudo-working electrode is a triggering electrode.

24. The electrode strip of Claim 21 wherein said pseudo-working electrode and said reference electrode pair are a resistance-measuring electrode pair.

25. A disposable electrode strip for detecting or measuring the concentration of an analyte in a fluid sample, said electrode strip comprising:

an insulating base strip having a first base end and a second base end;

a conductive layer disposed on one side of said base strip and scribed to delineate a pattern of three electrically-distinct conductive paths;

a middle insulator sized smaller than said insulating base strip and overlaying a substantial portion of said conductive layer, said middle

insulator having a cutout portion spaced from said first base end and exposing a limited area of said three conductive paths;

an electrode material comprising an enzyme, a redox mediator, a stabilizer, a binder, a surfactant, and a buffer, said electrode material being disposed in said cutout portion; and

a covering insulator sized to fit over and be coextensive with said middle insulator creating a sample fluid channel, said covering insulator having a covering insulator aperture spaced from said first base end and configured to expose at least a small portion of said cutout portion of said middle insulator.

26. The strip of Claim 25 wherein said sample fluid channel has a volume of about 0.22 microliters.

27. The strip of Claim 25 wherein said sample fluid channel is hydrophilic.

28. The strip of Claim 25 wherein said redox mediator is at least one metal complex selected from the group consisting of ferrocene, ferrocene derivatives and potassium ferricyanide, said stabilizer is a polyalkylene glycol, said binder is a cellulose material, said surfactant is a polyoxyethylene ether, and said buffer has a pH of about 5 to about 6.

29. The strip of Claim 28 wherein said mediator is potassium ferricyanide, said stabilizer is polyethylene glycol, said binder is methyl cellulose, said surfactant is t-octylphenoxypolyethoxyethanol, and said buffer is a citrate buffer.

30. The strip of Claim 29 wherein said electrode material is made of a mixture having starting components comprising about 6.5wt% of said potassium ferricyanide, about 2.5wt% of said polyethylene glycol, about 1wt% of said methyl cellulose, and about 0.03wt% of said t-

octylphenoxypolyethoxyethanol, and about 1wt% of said enzyme in said citrate buffer.

31. The strip of Claim 30 wherein said enzyme is glucose oxidase.

32. The strip of Claim 25 wherein said insulating base strip, said middle insulator, and said covering insulator are made from a plastic material selected from the group consisting of polyvinyl chloride, polycarbonate, polysulfone, nylon, polyurethane, cellulose nitrate, cellulose propionate, cellulose acetate, cellulose acetate butyrate, polyester, acrylic, and polystyrene.

33. The electrode strip of Claim 25 wherein said sample fluid channel contains a working electrode, a pseudo-working electrode and a reference electrode.

34. The electrode strip of Claim 33 wherein said pseudo-working electrode is a counter electrode.

35. The electrode strip of Claim 33 wherein said pseudo-working electrode is a triggering electrode.

36. The electrode strip of Claim 33 wherein said pseudo-working electrode and said reference electrode pair are a resistance-measuring electrode pair.

37. A method of making multiple, disposable sensors wherein each sensor has at least a working electrode, a reference electrode, a pseudo-working electrode, and a reagent matrix, wherein said reagent matrix contains an enzyme capable of catalyzing a reaction involving a substrate for the enzyme, said working electrode and said reference electrode being disposed in a fluid sample channel for measuring a fluid sample, said method comprising:

obtaining a base strip of an insulating material having a layer of conductive material disposed thereon, said base strip having a first edge and a second edge;

scribing in said conductive material a plurality of lines in a repetitive pattern wherein said plurality of lines contain a repetitive pattern capable of forming three conductive paths in each of said repetitive pattern;

disposing a middle layer of insulating material over said base strip, said middle layer having a repetitive pattern of an elongated cutout wherein each cutout of each of said repetitive pattern exposes an electrode portion of each of said three conductive paths of each repetitive pattern wherein said repetitive pattern of said elongated cutout are spaced from said first edge of said base strip, and wherein said middle layer is sized to expose a contact portion of each of said two conductive paths of each repetitive pattern for a distance from said second edge of said base strip;

disposing an reagent material into each elongated cutout of said repetitive pattern;

drying said reagent material at a temperature and for a length of time sufficient to solidify said reagent material in each of said elongated cutout;

disposing a top layer of insulating material over and coextensive with said middle layer, said top layer having a plurality of vent openings in a repetitive pattern wherein each of said vent openings exposes a portion of a corresponding repetitive pattern of said elongated cutout of said middle layer furthest from said first edge of said base strip, said base strip, said middle layer and said top layer forming a laminated strip;

cutting along and parallel to said first edge of said laminated strip a predetermined distance creating a sample inlet port in each of said elongated cutout for each of said repetitive pattern;

cutting along and parallel to said second edge of said laminated strip a predetermined distance creating two separate contacts for each of said repetitive pattern; and

separating each of said repetitive pattern at predetermined intervals along said laminated strip.

38. The method of Claim 37 wherein said drying step further includes heating said reagent material at a temperature of about 55 °C.

39. The method of Claim 38 wherein said drying step further includes heating said reagent material for about two minutes.

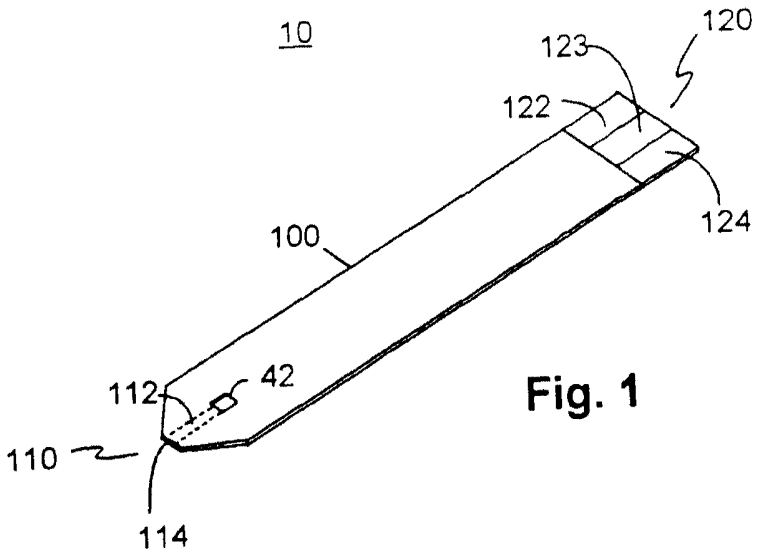

10
120
123
122
124
100
42
112
110
114

Fig. 1

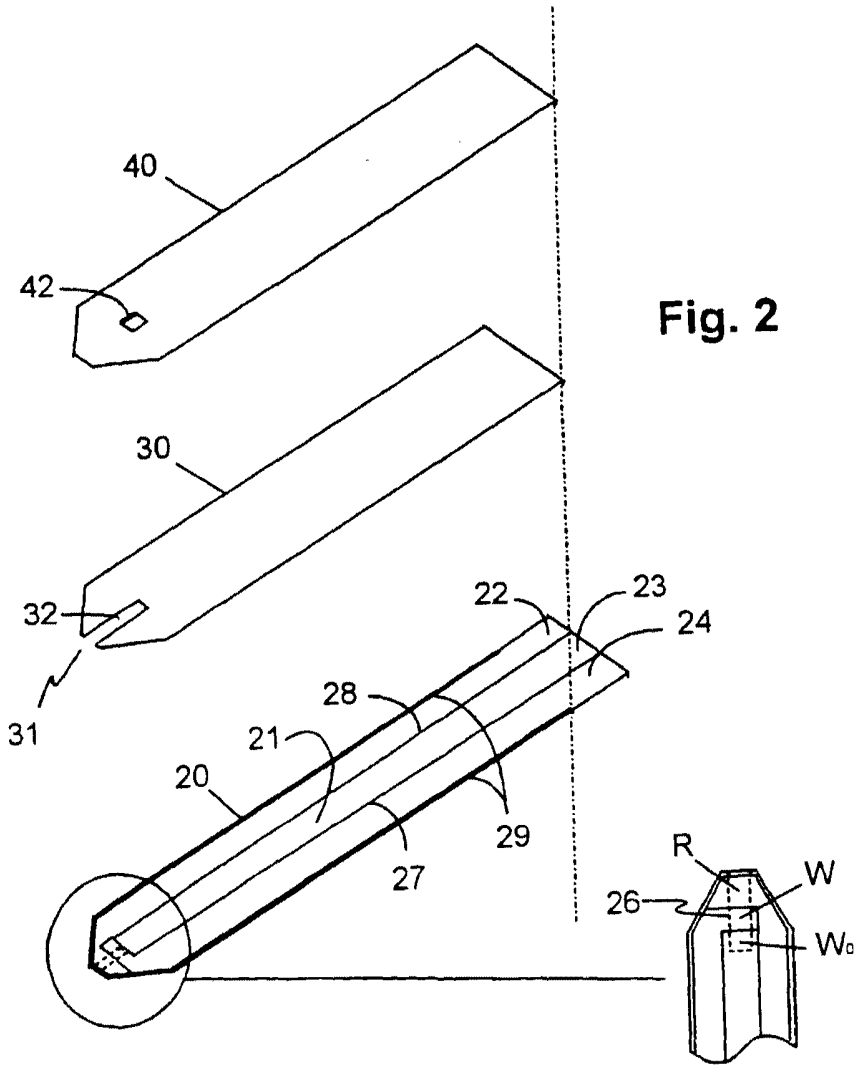

40
42
30
32
31
22
23
24
28
21
20
29
27
R
W
26
W0

Fig. 2

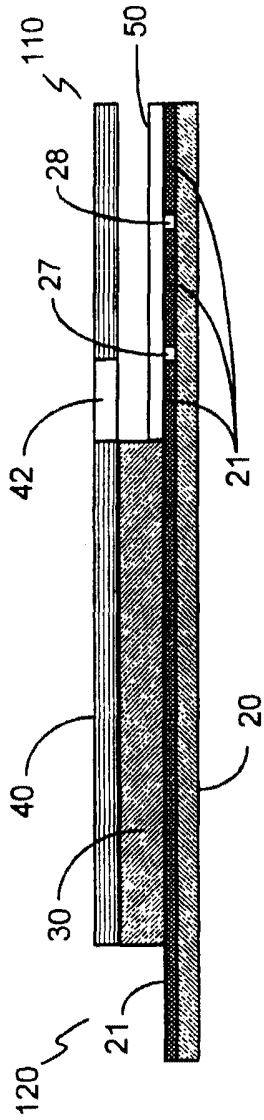


Fig. 3

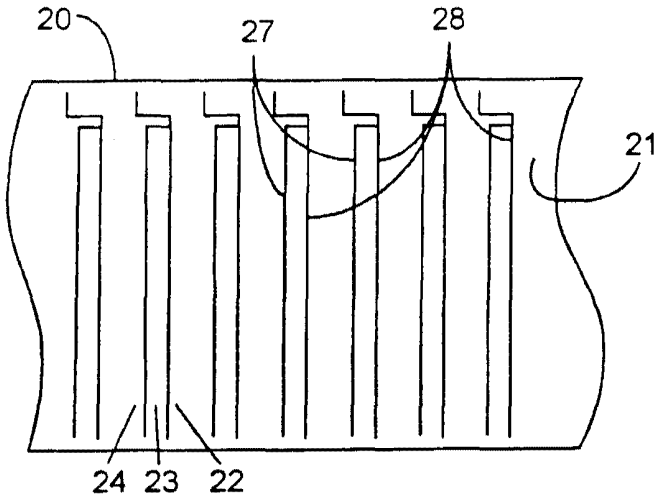


Fig. 4 A

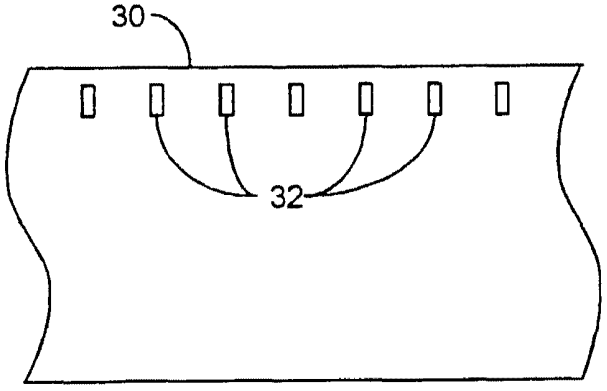


Fig. 4 B

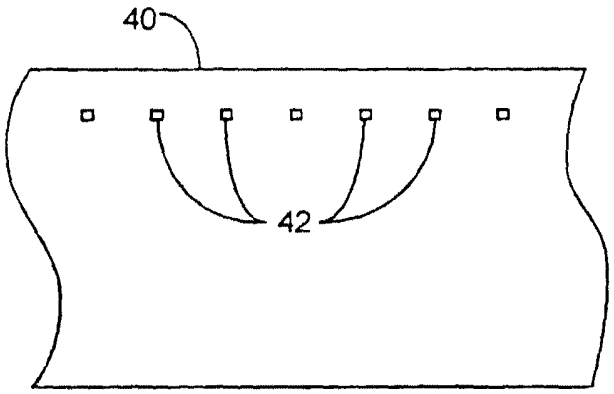


Fig. 4 C

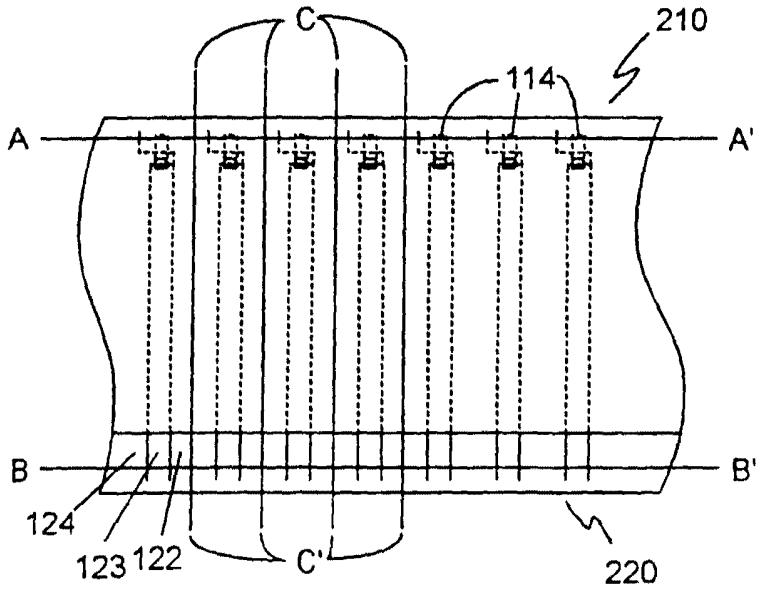


Fig. 4 D

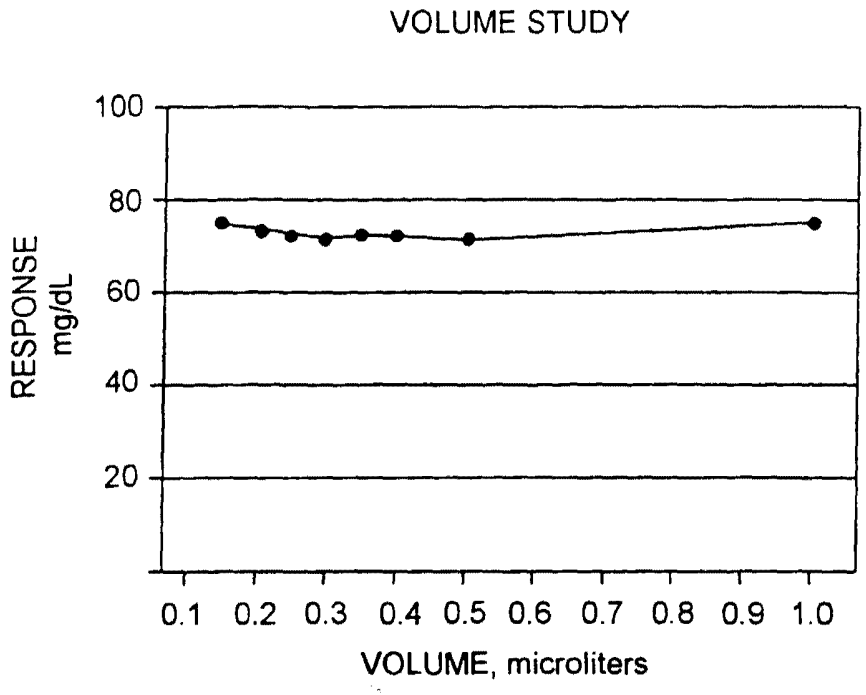

VOLUME STUDY
RESPONSE mg/dL
100
80
60
40
20
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
VOLUME, microliters

Fig. 5

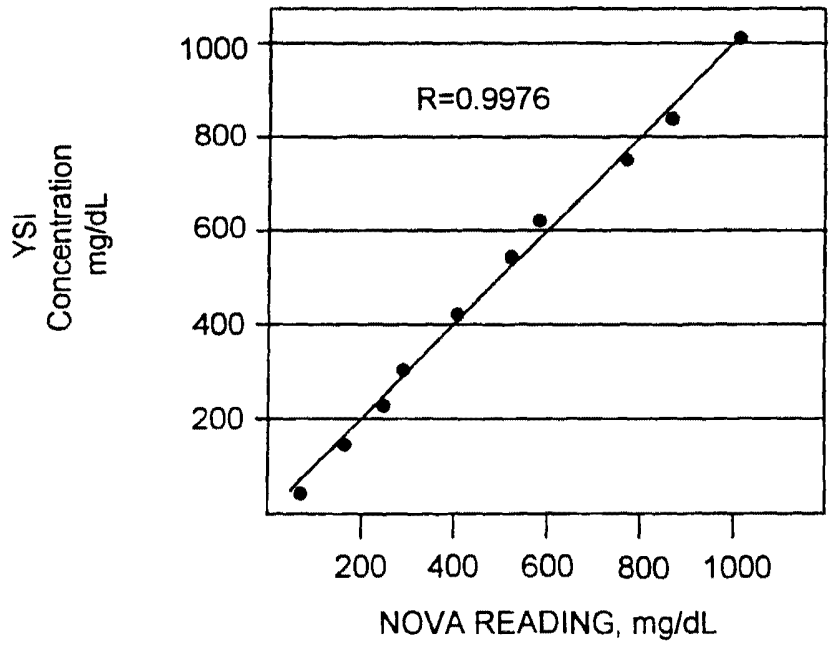

YSI Concentration mg/dL
R=0.9976
1000
800
600
400
200
200 400 600 800 1000
NOVA READING, mg/dL

Fig. 6

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/US 00/15106

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/327 C12Q1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q A61B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, COMPENDEX, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 11, 28 November 1997 (1997-11-28) & JP 09 189675 A (MATSUSHITA ELECTRIC IND CO LTD), 22 July 1997 (1997-07-22) abstract ---	1-8, 21-24, 37-39
X	US 5 437 999 A (DIEBOLD ERIC R ET AL) 1 August 1995 (1995-08-01) column 1, line 66 -column 2, line 33 column 3, line 29 - line 45 column 12, line 35 -column 13, line 8 table 1 -----	1-9,11, 12,17, 20, 25-27, 31,32, 37-39

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 September 2000	22/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muñoz, M

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna l Application No
PCT/US 00/15106

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 09189675 A	22-07-1997	US 6004441 A	21-12-1999
US 5437999 A	01-08-1995	CA 2183865 A	24-08-1995
		EP 0753051 A	15-01-1997
		JP 9509740 T	30-09-1997
		WO 9522597 A	24-08-1995

OCT 12 2001
PATENT & TRADEMARK OFFICE

Docket No. RDID 00108 US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: BHULLAR et al.

Serial No.:09/942,515 Filed: August 29, 2001

For: BIOSENSOR

Assistant Commissioner for Patents
Office of Initial Patent Examination
Customer Service Center
Washington, DC 20231

REQUEST FOR CORRECTED FILING RECEIPT

1. Attached is a copy of the official Corrected Filing Receipt received from the USPTO in the above application for which issuance of a corrected Filing Receipt is respectfully requested with the error marked in green.

2. There is an error with respect to the following, which is incorrectly entered:

Error in	*Corrected data*
Applicant name	James L. Pauley JR., Fishers, IN.

3. A copy of the Declaration filed in the USPTO reflecting the correct spelling of the inventor's name is enclosed.

4. The correction is not due to any error by applicant and no fee is due.

Oct. 9, 2001
Date

Jill L. Woodburn
Signature
Jill L. Woodburn, Reg. 39,874
The Law Office of Jill L. Woodburn, L.L.C.
6633 Old Stonehouse Dr.
Newburgh, IN 47630
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OCT 12 2001

UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED Oct. 5, 2001

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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/942,515	08/29/2001	1743	1006	RDID 00108US	4	32	4

CONFIRMATION NO. 8155

FILING RECEIPT

OC000000006840693

Jill L. Woodburn
The Law Office of Jill L. Woodburn, L.L.C.
6633 Old Stonehouse Dr.
Newburgh, IN 47630

Date Mailed: 10/03/2001

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Raghbir S. Bhullar, Indianapolis, IN;
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John T. Austera, Indianapolis, IN;
Douglas P. Walling, Indianapolis, IN;
Timothy I. Ranney, Lebanon, IN;
James L. ~~Pauley~~ Pauley JR., Fishers, IN;

OCT 15 2001

Domestic Priority data as claimed by applicant

Foreign Applications

If Required, Foreign Filing License Granted 10/03/2001

Projected Publication Date: 03/06/2003

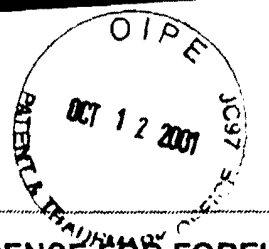
Non-Publication Request: No

Early Publication Request: No

Title

Biosensor

Preliminary Class
204



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Title 37, Code of Federal Regulations, 5.11 & 5.15

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Express Mail Label No. ET471793701US



Docket No - RDID 00108 US

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

BIOSENSOR

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International Application Number _____

and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined, in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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JAN 27 2003 JC26 PATENT & TRADEMARK OFFICE

PTO/SB/21 (08-00)
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/942,515
Filing Date	08/29/2001
First Named Inventor	Bhullar
Group Art Unit	1743
Examiner Name	Unknown
Total Number of Pages in This Submission	3+Ref.
Attorney Docket Number	RDID 00108 US

ENCLOSURES *(check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment / Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☑ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Assignment Papers *(for an Application)*
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
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- ☐ After Allowance Communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) *(please identify below)*: 1 reference

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	The Law Office of Jill L. Woodburn, L.L.C. Jill L. Woodburn
Signature	
Date	01/21/2003

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CIPE JC26 JAN 27 2003 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Bhullar et al.

Application No.: 09/942,515 Group No.: 1743

Filed: 29 August 2001 Examiner: ~~Unknown~~ IVOJ CERCA

For: Biosensor

TECH

Supplemental Information Disclosure Statement

Assistant Commissioner for Patents
Washington, D.C. 20231

This supplemental statement is filed in the application identified above pursuant to 37 C.F.R.§1.56 and in compliance with the requirements of 37 C.F.R. §1.98.

The Examiner's attention is called to the reference listed on the attached form, which was cited in the European Search Report mailed on December 20, 2002, for a corresponding application. No representation is intended that a complete search has been made of the prior art or that better art references are not available. The filing of this supplemental statement shall not be construed to be an admission that the information cited in this statement is, or is considered to be, material to patentability as defined in §1.56 (b).

It is believed that no fees are due with submission of this supplemental disclosure statement. If however, fees are due, please change all fees due in connection with this supplemental disclosure statement to Deposit Account No. 02-2958 with reference to (RDID 00108 US).

Respectfully submitted,
The Law Office of Jill L. Woodburn, L.L.C.

Date

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OIPE JC29 JAN 27 2003 PATENT & TRADEMARK OFFICE

U.S. Department of Commerce Patent and Trademark Office	ATTY DOCKET NO.	SERIAL NO.
	RDID 00108	09/942,515
	APPLICANT	
INFORMATION DISCLOSURE STATEMENT	Bhullar et al.	
	FILING DATE 29-Aug-01	GROUP 1743

*Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes No
	AL	2 311 614 A	10-01-97	GB	G01N27	327	--
	AM						
	AN						
	AO						
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OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, etc.)

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Examiner	Date Considered

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

(12) UK Patent Application (19) GB (11) 2 311 614 (13) A

(43) Date of A Publication 01.10.1997

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(51) INT CL[6]
G01N 33/543 33/487 35/00

(52) UK CL (Edition O)
G1N NBMA N25B N25DX
U1S S1296

(56) Documents Cited
EP 0359049 A EP 0352689 A WO 95/02189 A
WO 94/19684 A WO 92/05449 A WO 90/01700 A
US 4974592 A

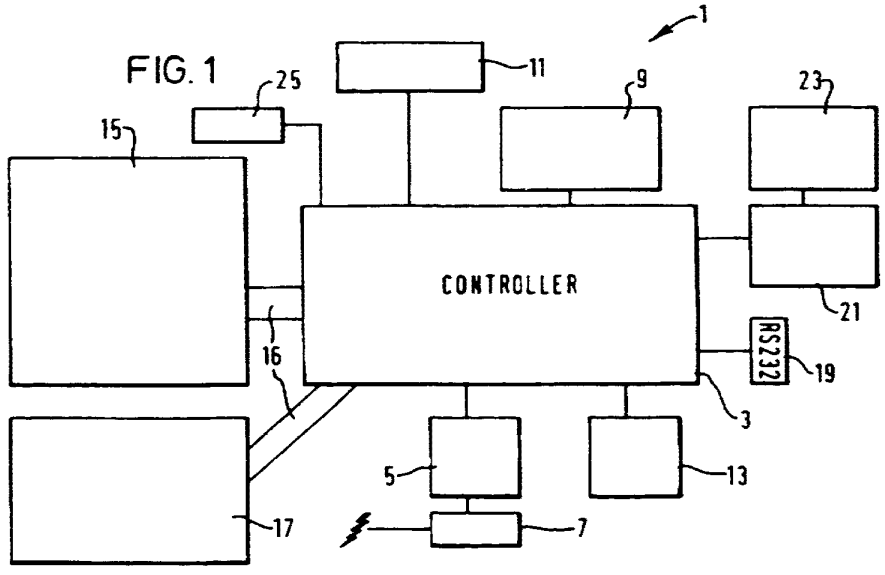
(58) Field of Search
UK CL (Edition O) G1N NBMA NBMX
INT CL[6] G01N 33/483 33/487 33/543 35/00
ONLINE: WPI

(74) Agent and/or Address for Service
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(54) Automatic diagnostic apparatus

(57) An automatic diagnostic apparatus 1 comprises a controller 3 for controlling operation of the apparatus 1 and for processing data, a sensing system 15 operably connected to the controller 3 for performing an assay of a sample and communicating data from said assay to the controller 3, and output means 11, 23 for communicating processed data to a user. Preferably the system includes voltage supply means for applying a potential difference to the sensing system 15, an electroimmunoassay biosensing system, and means for generating flow of a sample through the sensing system. Preferably the apparatus uses a sample holder in the form of a container with two bases, one raised above the other with the raised base having a depression in it, and a centrifuge for spinning the sample holder so that a sample with lighter and heavier components is separated with the lighter component being retained in the depression. The apparatus can receive reagent cartridges with identifying labels in the form of barcodes. It can be used to monitor acute myocardial infarction.

FIG. 1



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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

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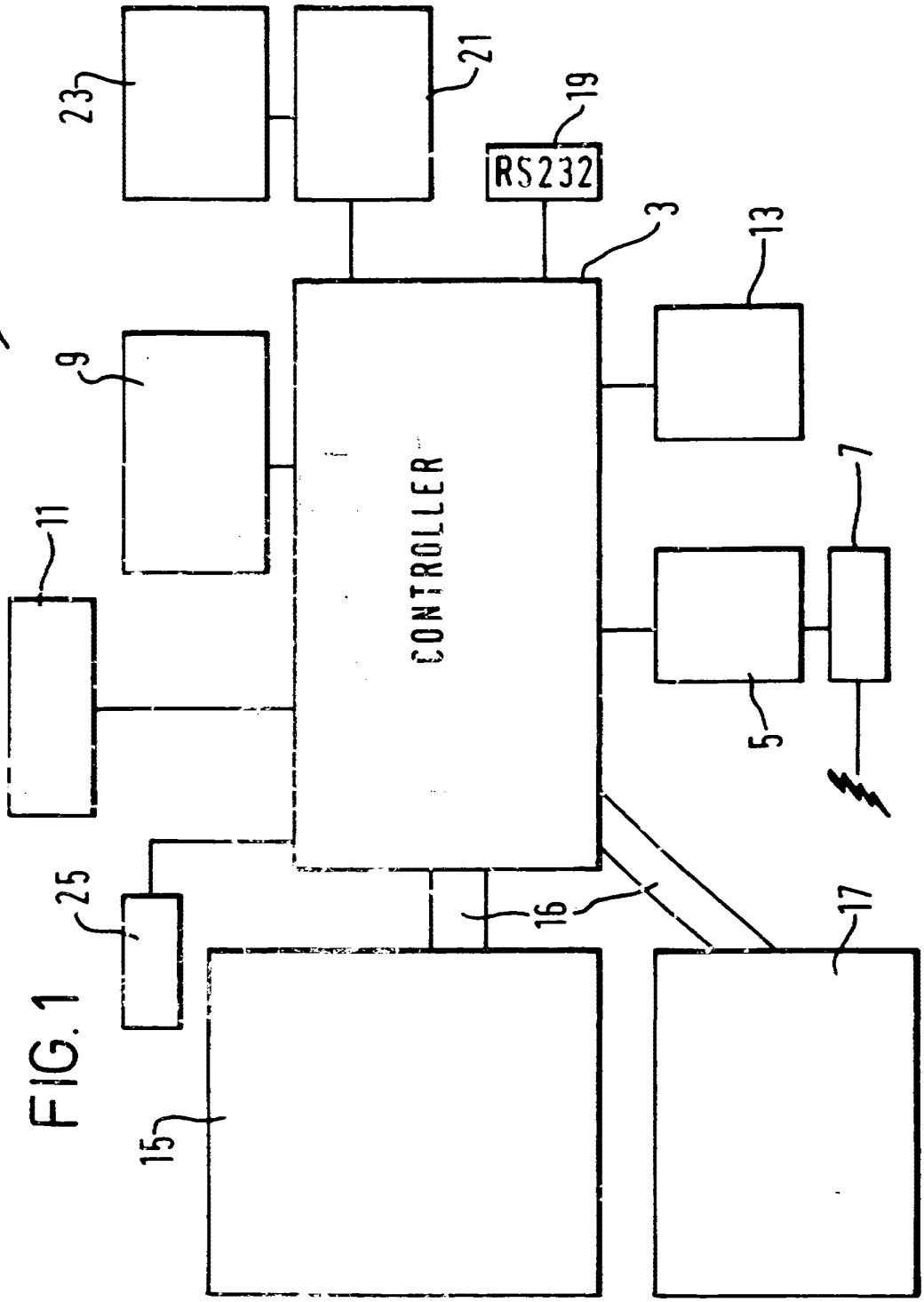


FIG. 1

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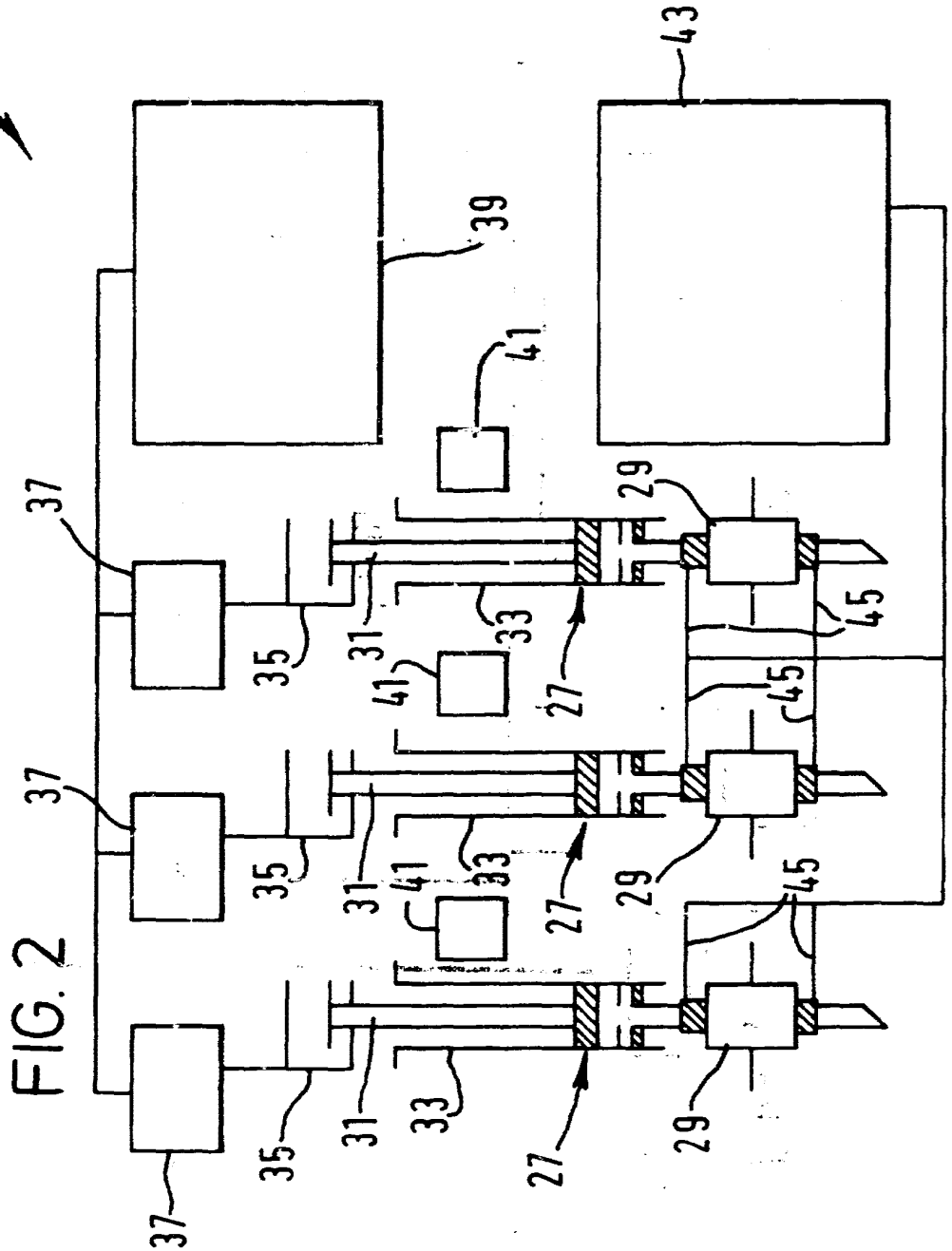


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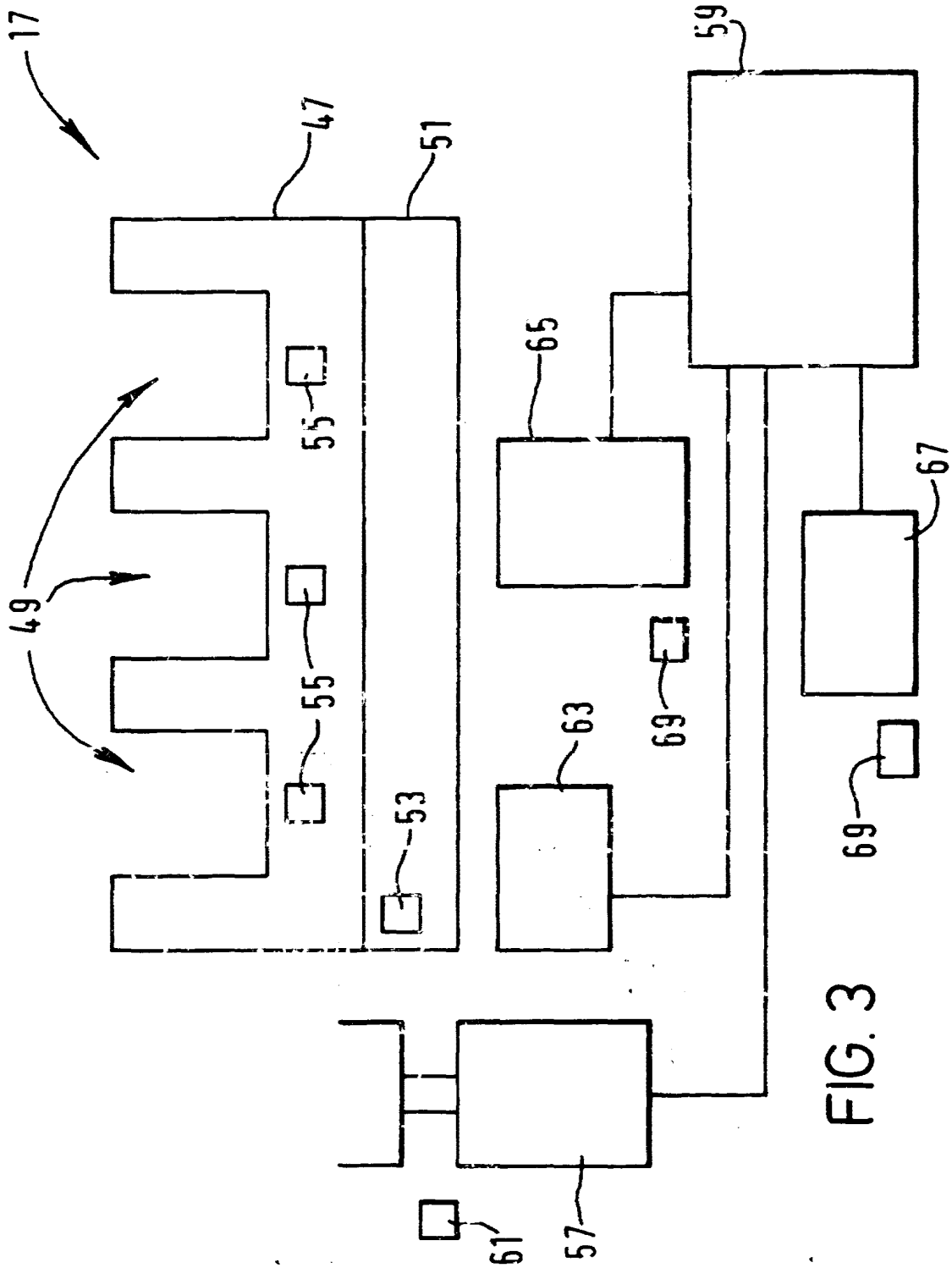


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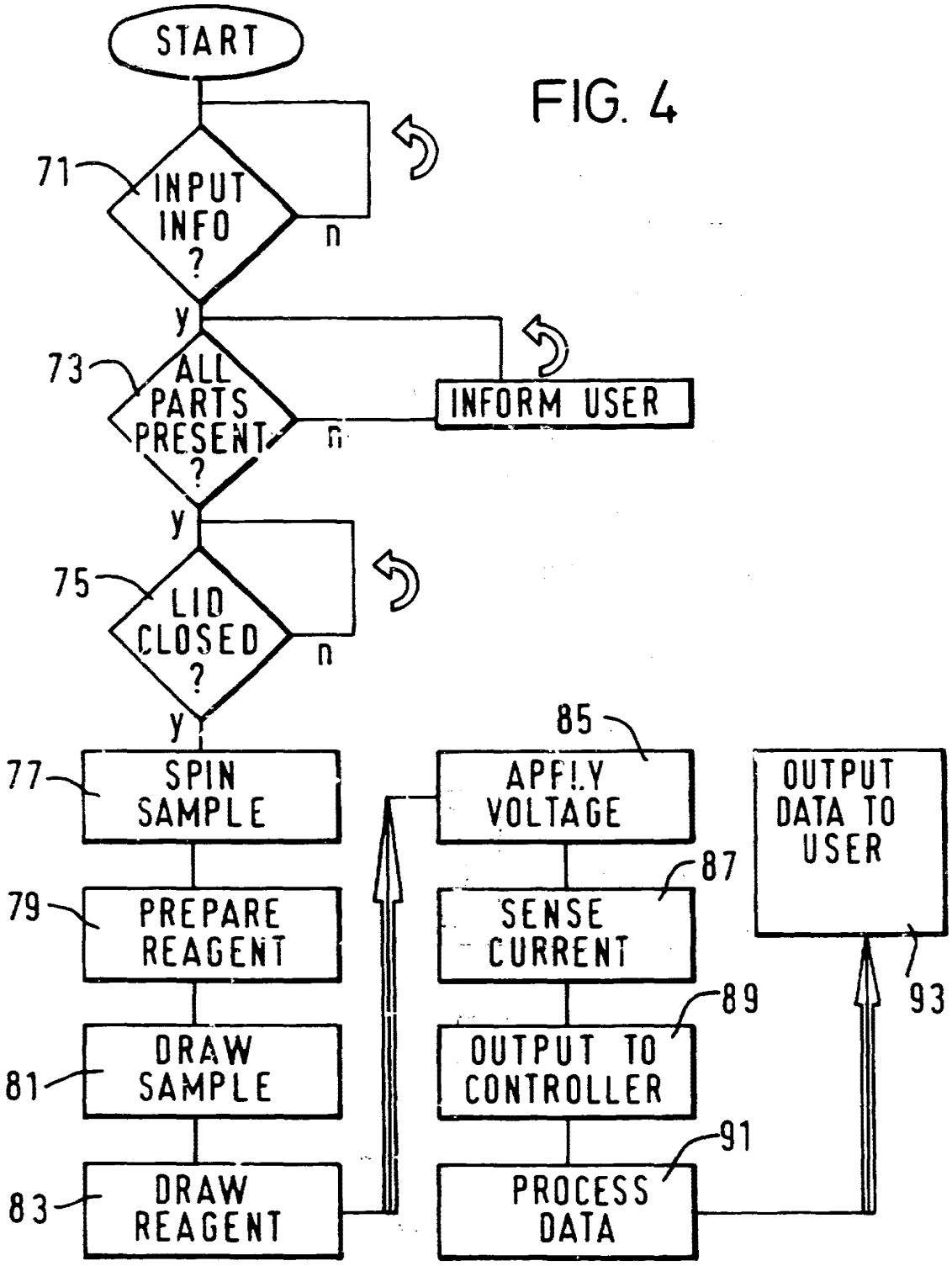


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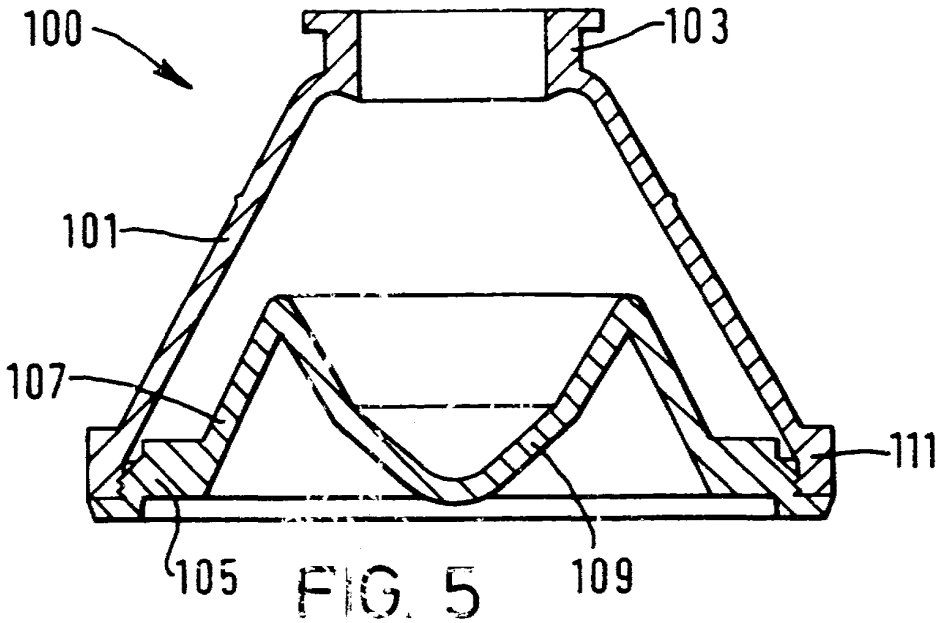


FIG. 5

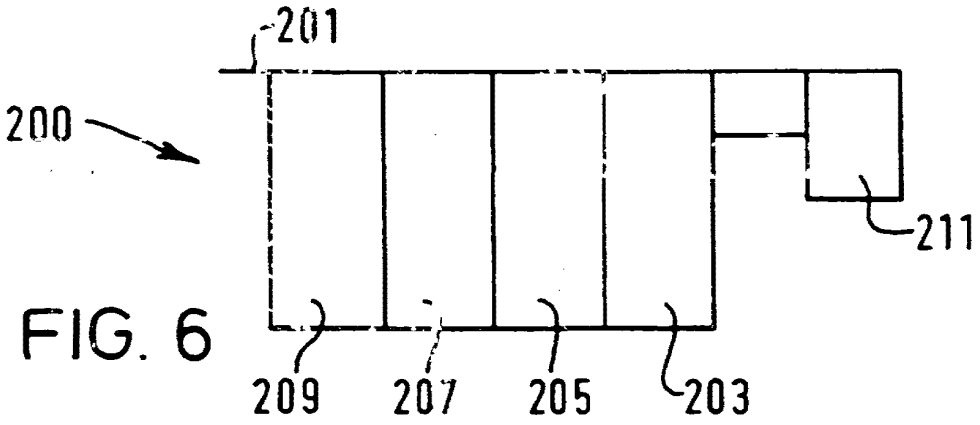


FIG. 6

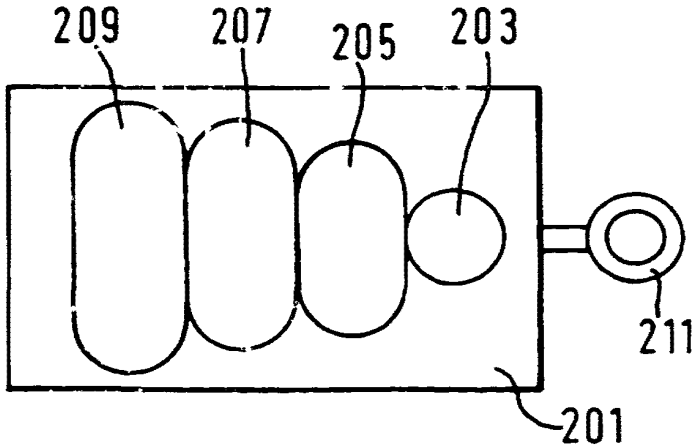


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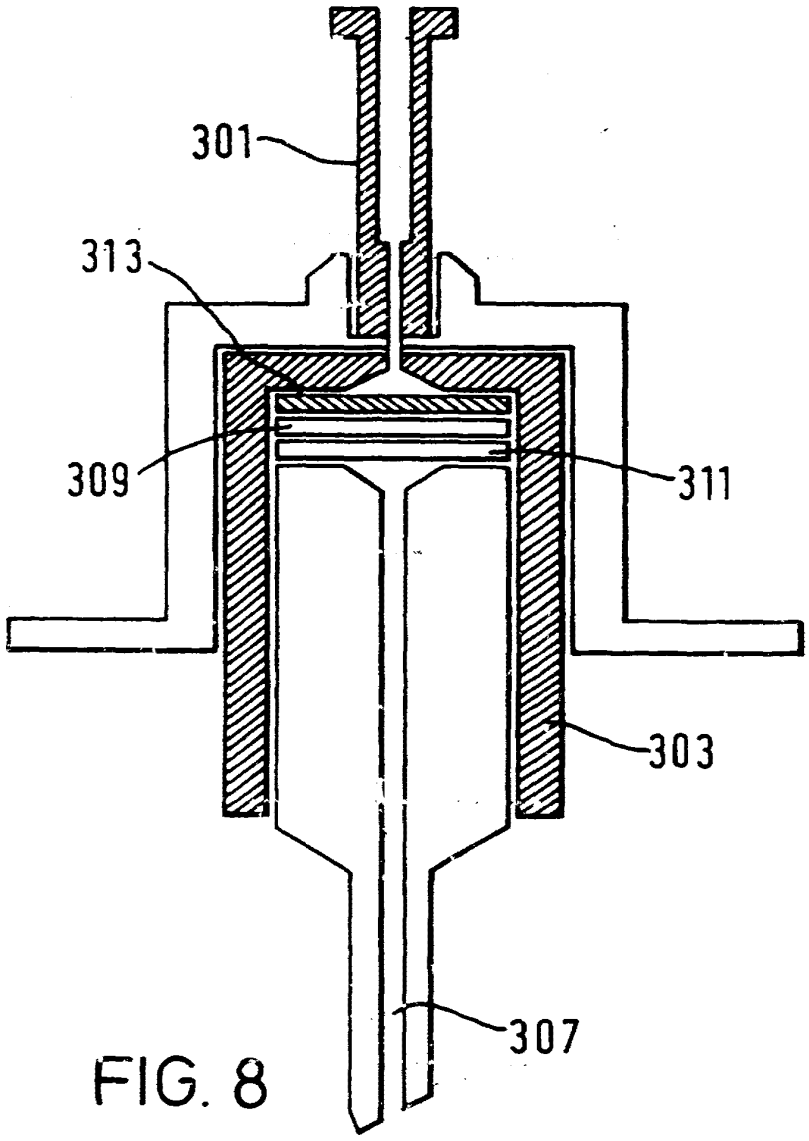
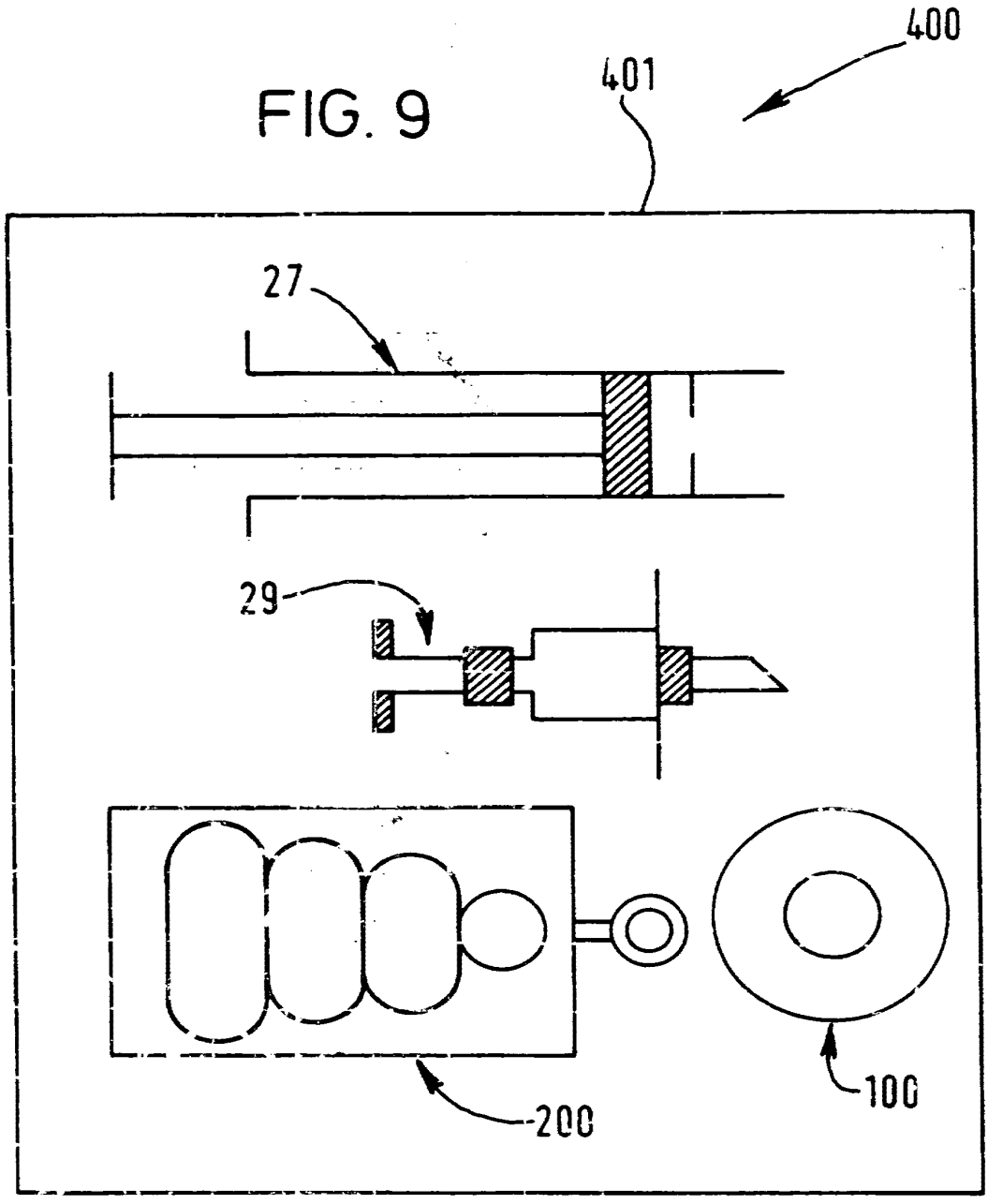


FIG. 8

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FIG. 9



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FIG. 10

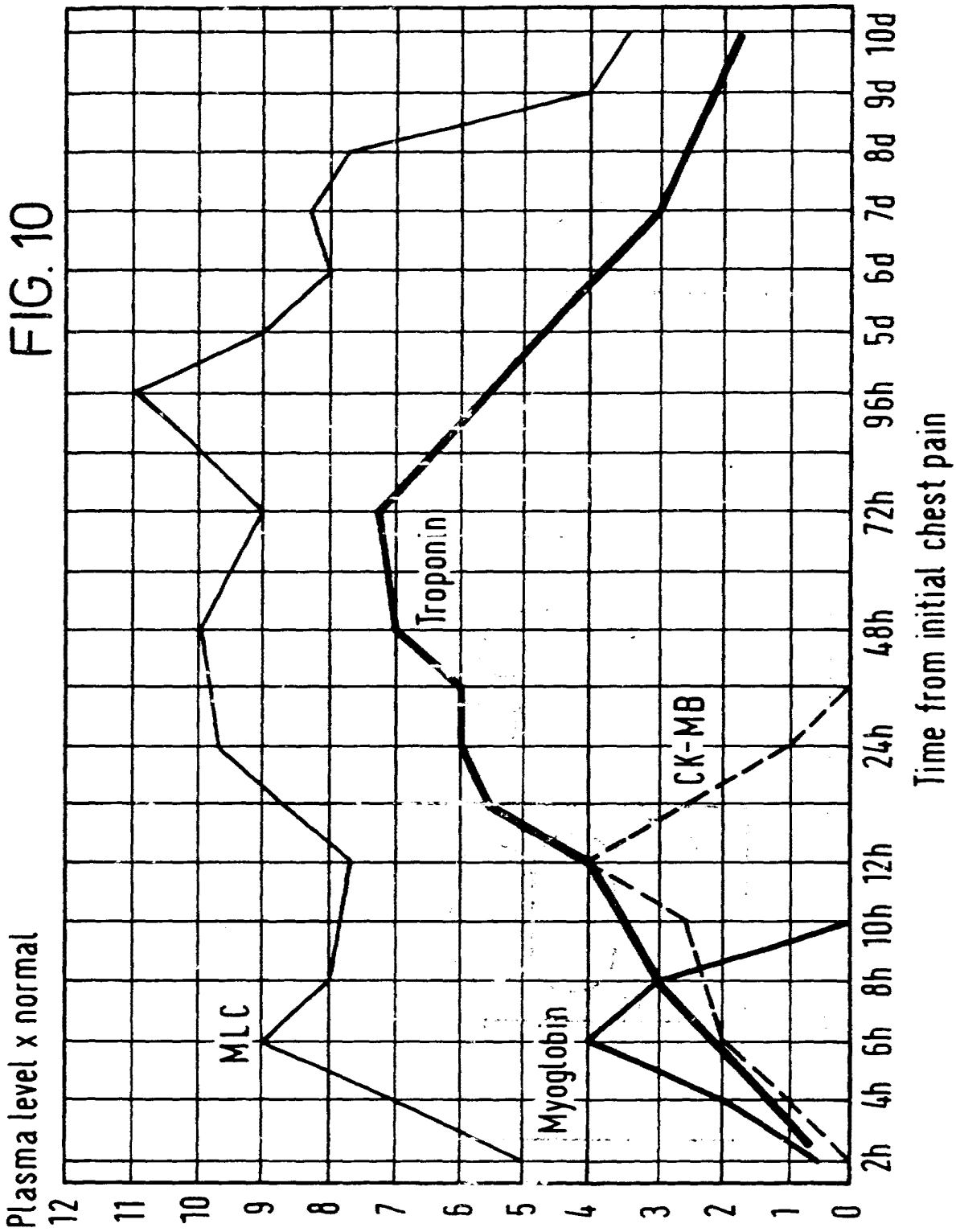
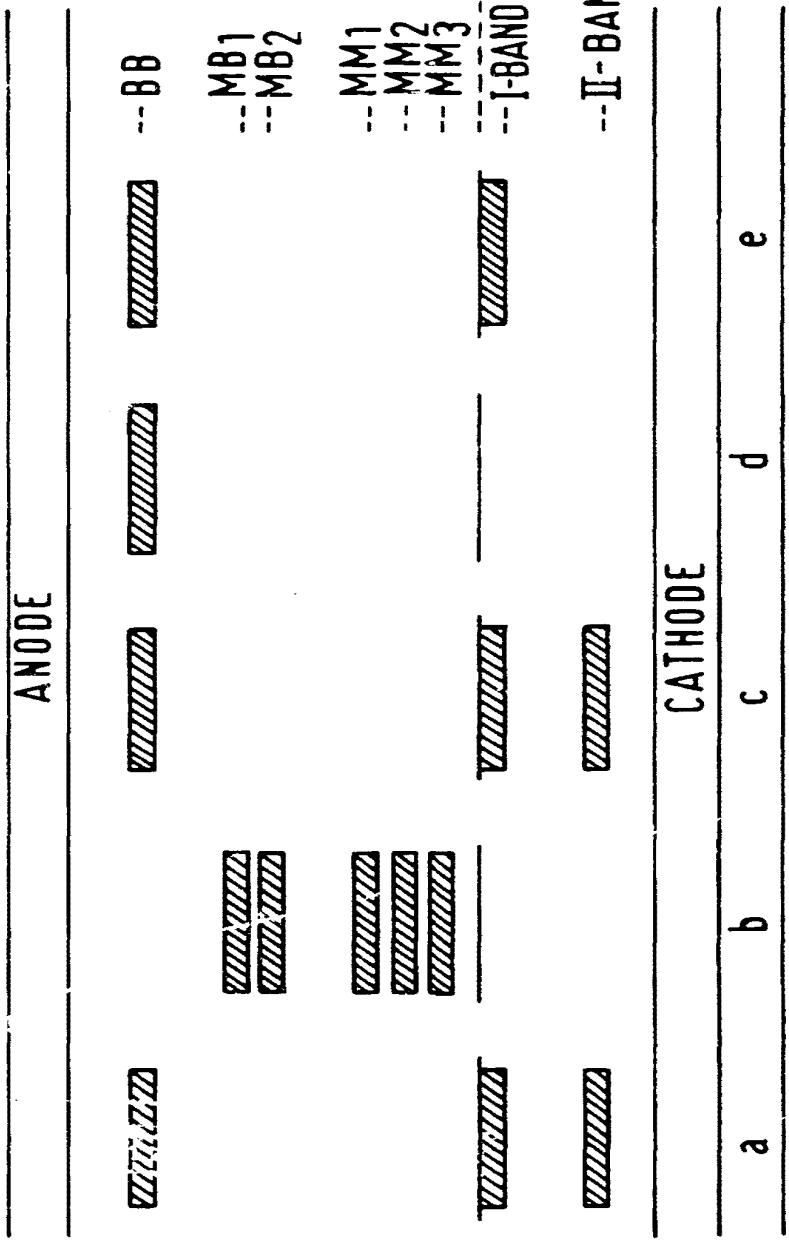

Plasma level x normal
0
1
2
3
4
5
6
7
8
9
10
11
12
MLC
Troponin
Myoglobin
CK-MB
2h
4h
6h
8h
10h
12h
24h
48h
72h
96h
5d
6d
7d
8d
9d
10d
Time from initial chest pain

FIG. 11



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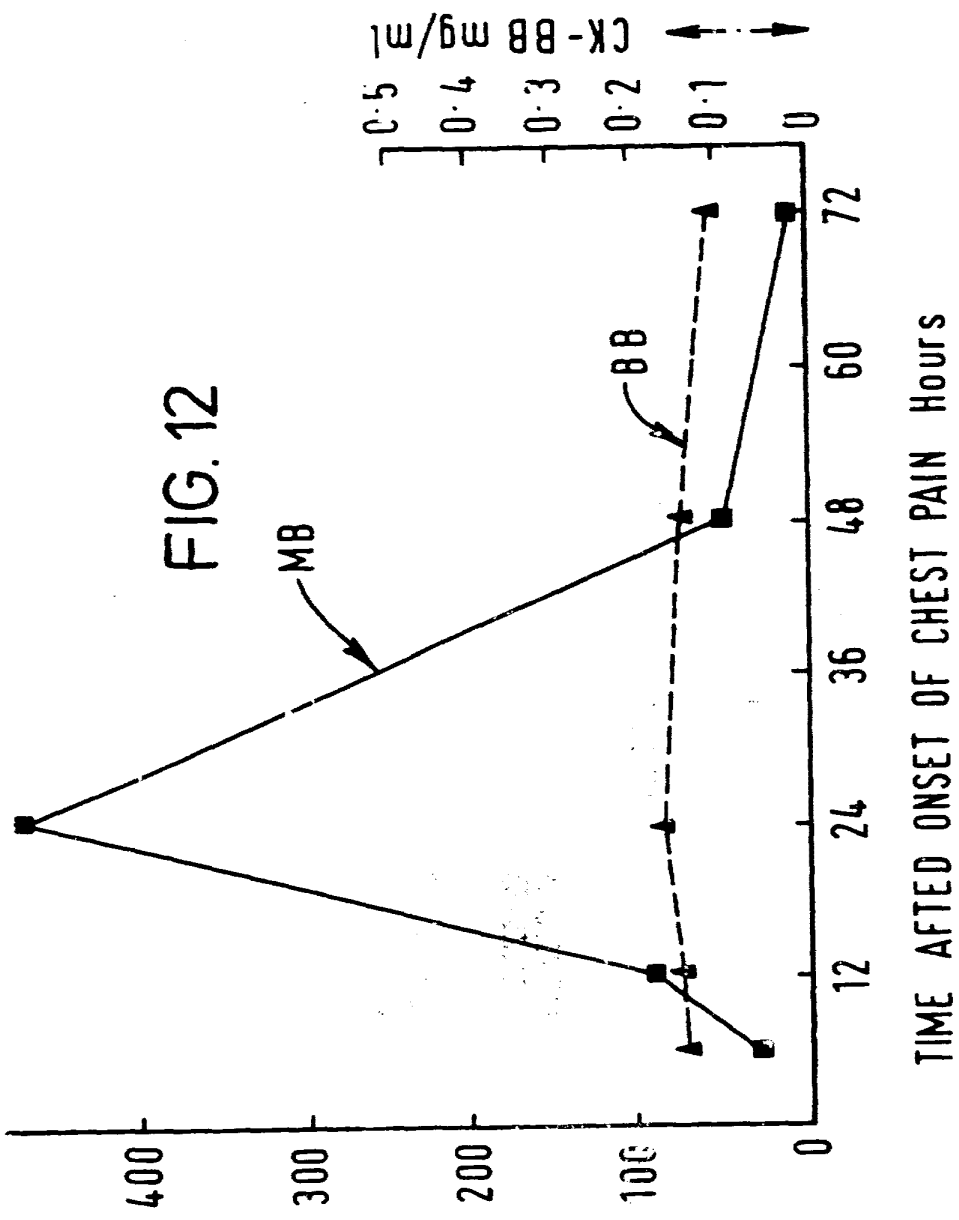


FIG. 12

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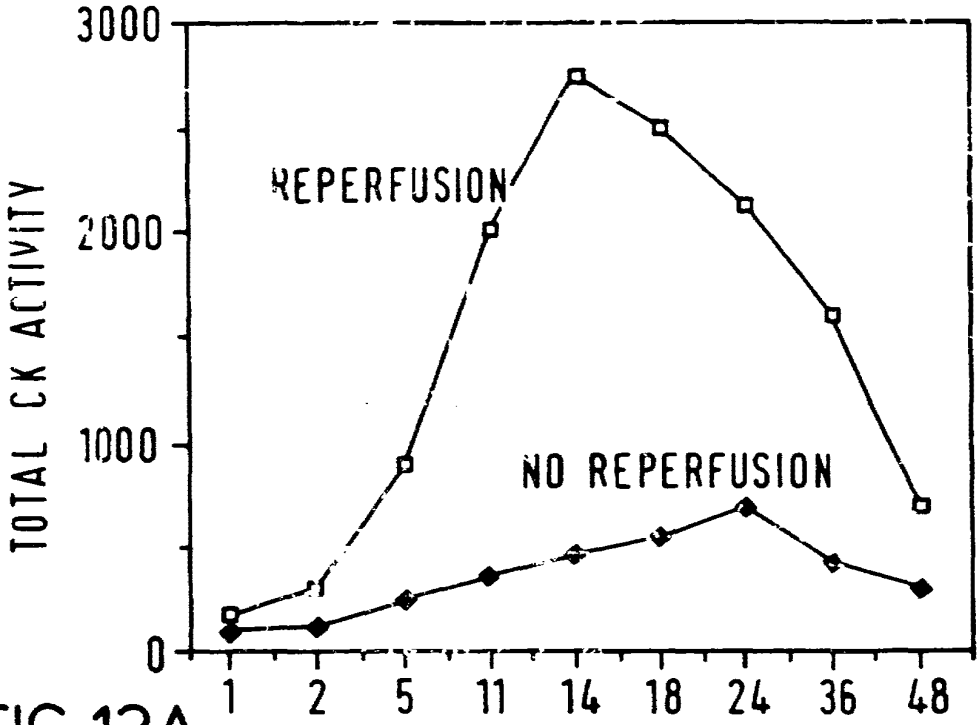


FIG. 13A

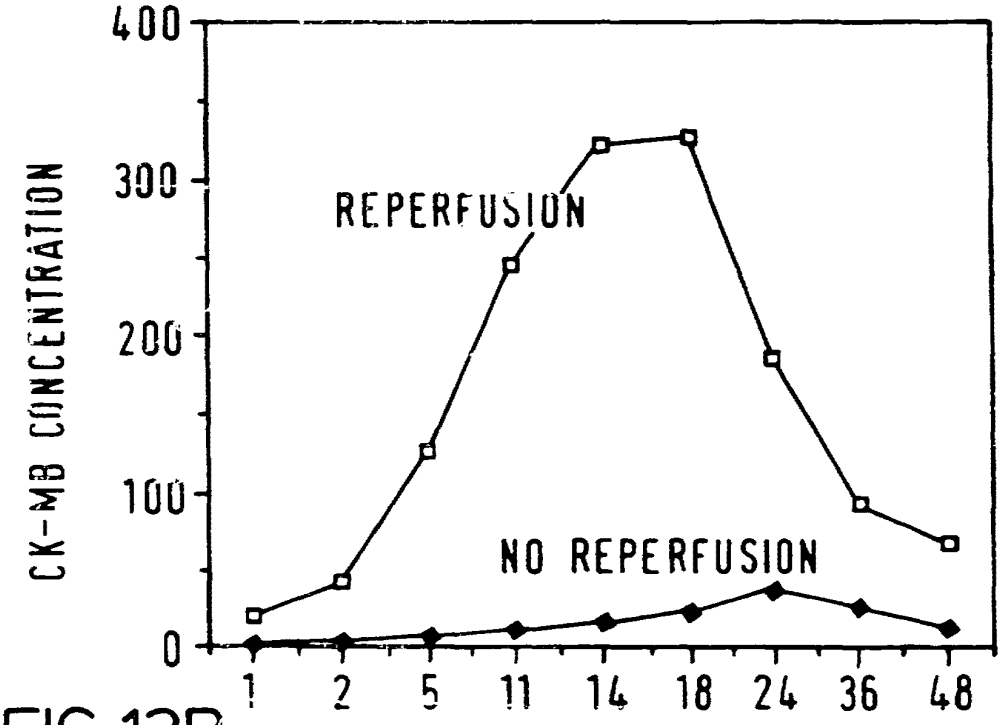


FIG. 13B

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FIG. 14

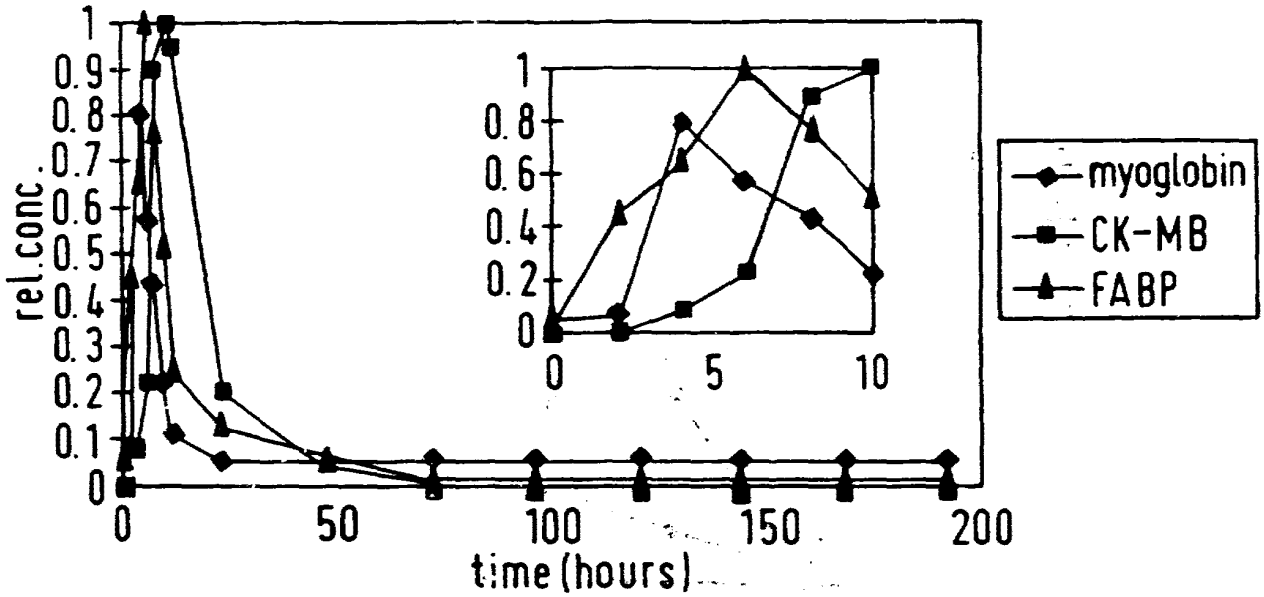
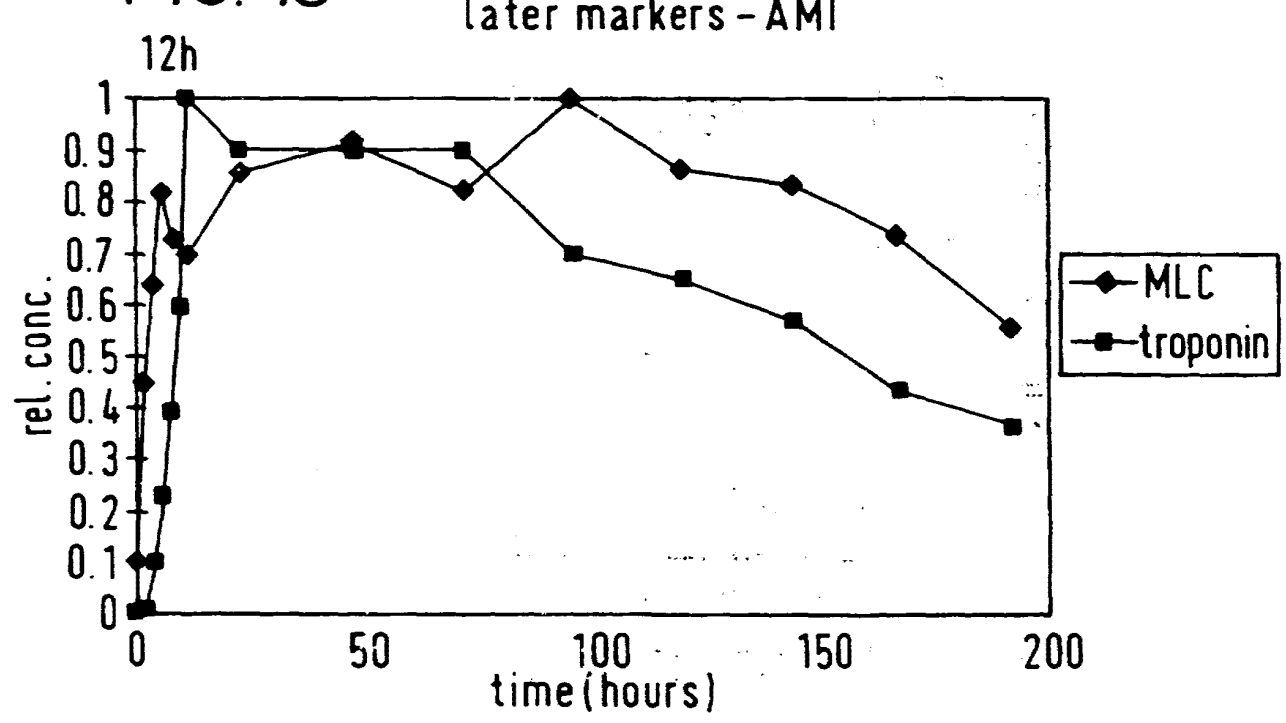


FIG. 15



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AUTOMATIC DIAGNOSTIC APPARATUS

This invention relates generally to an automatic diagnostic apparatus.

When a patient is treated by a physician, it is not uncommon for the physician to take samples of body fluids to be sent on to a laboratory for analysis. The testing often has to be done manually and thus, inevitably, some delay is incurred in the processing of these samples which also delays the point at which the results can be communicated to the patient.

Even in Hospitals, where the condition of the patients can be extremely serious, the samples still have to be sent away to an "in-house" laboratory for testing. It can often take a matter of hours for the results of these tests to be communicated to the physician in charge of that patient. Accordingly, it is not uncommon for the physician to begin treating a patient without knowing the results of any requested testing.

In situations where the patient is seriously ill, the delay incurred in testing samples could conceivably put the well-being of that patient at risk.

One might consider that a suitable way to overcome this problem would be for the physician in charge of a particular patient to conduct the testing himself/herself, without sending the samples away to a laboratory. However, the testing of samples is often a complex process which must be carried out by highly skilled personnel if the results are to be reliable and hence of any real use to the physician.

Therefore, there is a need in the art for an apparatus which can be quickly and reliably operated by a user (who will sometimes referred to as an operator) to test samples, particularly samples obtained from patients.

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In accordance with the present invention, there is provided an automatic diagnostic apparatus comprising: a controller for controlling operation of the apparatus and for processing data; a sensing system operably connected to the controller for performing an assay, preferably an electrochemical assay (more preferably an electrochemical immunoassay), of a sample and communicating data from said assay to said controller; optionally voltage supply means for applying a potential difference to said sensing system; and output means for communicating processed data to a user.

The present invention therefore provides an automated apparatus for the testing of samples, especially patient samples. If patient samples are tested then the results of this testing can be made available to a physician within a matter of minutes and thus provide an early and rapid diagnosis of a patient's condition.

In accordance with the present invention, there is also provided a method of automatic diagnosis, the method comprising the steps of:

(a) placing a sample within an automatic diagnostic apparatus;

(b) optionally generating instructions with a controller for instructing a voltage supply means to apply a voltage to a sensing system;

(c) controlling said sensing system with said controller to perform an assay, preferably an electrochemical assay (more preferably an electrochemical immunoassay), of said sample and to generate data for output to said controller;

(d) processing said data in said controller to generate processed data; and

(e) outputting said processed data to a user.

The automatic diagnostic apparatus, and the method of operating the same, is particularly useful for the testing of acute myocardial infarction and for the monitoring of reperfusion.

Accordingly, in accordance with a preferred embodiment of the present invention there is provided a method of automatically diagnosing myocardial infarction, the method comprising the steps of: monitoring *ex vivo* levels of one or more detectable cardiac marker proteins, such as any one or more of CK, CK-MM, CK-MB,

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myoglobin, cardiac myosin light chain(s), Troponin T or Troponin I, or a cardiac marker suitable for the diagnosis of acute myocardial infarction. Advantageously, this method enables a quantitative assay to be conducted for these protein combinations.

Preferably the above method is accomplished with the above mentioned apparatus.

However, it will of course be understood, that whilst the present invention is preferably used for diagnostic testing for myocardial infarction, other testing (such as for any other clinical condition) may alternatively be conducted. Thus, the present disclosure is not to be read as being limited to the diagnostic testing of myocardial infarction only.

Prior to the testing of a patient's condition, it is often necessary to separate the sample from the patient into its constituent components. This separation is usually accomplished by placing the sample in a test tube, for example, and spinning the test tube at high speed in a centrifuge.

Throughout the spinning process, the sample separates into its constituent components with the heavier components moving towards the bottom of the test tube and the lighter components moving towards the top of the test tube. For example, if a sample of blood is taken and spun as described above, the heavier red blood cells move towards the bottom of the tube and the lighter plasma moves towards the top of the test tube.

The required portion of the sample may then be removed from the test tube. However, the operator must be careful to ensure that the tube is not subject to any further agitation, as such agitation may cause the components to recombine. The operator must also be careful to ensure that when he/she withdraws the required component of the sample, that component is not contaminated with any of the other component in the tube. Thus the withdrawing of separated components from a spun sample can be problematic.

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In accordance with the present invention, there is also provided a container having a first base and a second base, said second base being raised from said first base and having a depression provided therein, such that when material comprising a heavier component and a lighter component is placed within said container and spun, said heavier component is forced towards said first base and said lighter component is forced towards and onto said second base and subsequently retained within said depression.

In this way, the lighter component of a separated material may be easily withdrawn from the depression by an operator (which may be a mechanical or an electromechanical operator). Furthermore, the risk of that operator accidentally contaminating the lighter component with the heavier component, either by agitating the container or accidentally withdrawing any of the heavier component, is significantly reduced.

United Kingdom Patent Application No. 9409449.7 (published as GB-A-2 289 339) discloses an electrochemical through-flow immunoassay biosensor. The biosensor comprises a solid phase immunoassay system, a porous working electrode, a counter electrode and a means for producing a fluid flow through the biosensor. Whilst this arrangement produces excellent results in the laboratory, it could suffer from a number of drawbacks when used in a clinical environment requiring rapid analysis of a number of samples. The most significant of these may be associated with the fact that the biosensor must be thoroughly cleaned before it can be used again, or used to test another sample. Conceivably, the biosensor could be thrown away immediately after use. However, the relatively expensive material from which the preferred biosensor body and preferred biosensor electrodes are manufactured could quickly make such a strategy uneconomic. In addition, the associated equipment used with the biosensor would still have to be thoroughly cleaned and so, any time saving attained by the disposal of the biosensor would be counteracted by the time needed to clean the associated equipment.

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In accordance with the present invention, there is also provided a disposable electrochemical immunoassay biosensor comprising: a sensor body with a depression therein and a sensor outlet in said depression; an apertured counter electrode provided in abutment with one side of said depression such that said counter electrode aperture communicates with said outlet; an apertured working electrode provided in abutment with another side of said depression such that said working electrode aperture communicates with said sensor outlet; an immunoassay system provided in close proximity to said working electrode, and an apertured sensor inlet means also provided within said working electrode and in communication with said immunoassay system; wherein said sensor body is manufactured from a plastics material and said working and counter electrodes are manufactured from an electrically conductive plastics material.

Preferably, the immunoassay system is provided within the working electrode.

Alternatively or additionally, at least one of the electrodes may include other conventional electrode materials, such as silver (Ag) / silver chloride (AgCl).

In this way, the biosensor of the present invention may be manufactured from relatively inexpensive materials and, thus, a new biosensor may be used for each test and the old biosensor may be disposed of. The use of such a biosensor removes the need for extensive time-consuming cleaning of the biosensor.

In accordance with another embodiment of the invention, there is also provided a conducting plastic electrode suitable for use in a diagnostic apparatus. The present invention also provides for use of a conducting plastic electrode for an electrochemical immunoassay.

In order to perform an electrochemical immunoassay with conventional techniques, the operator would first have to prepare a suitable reagent. The preparation of this reagent may be a relatively complex process that would probably have to be repeated on each occasion that a diagnostic test was to be undertaken. By way of example,

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for a physician operating in his/her surgery, the preparation of suitable reagents would require the physician to keep stocks of necessary chemicals and to waste valuable time making up suitable reagents.

Furthermore, each preparation of a suitable reagent by the physician may be subject to minor variations that could cause doubt to be cast on tests made on the same patient, but with different sets of reagents.

Also, if a physician were to prepare a number of different reagents for use with different diagnostic tests, it is conceivable that these reagents could become contaminated with each other or, more seriously, one reagent could be mistaken for another.

Thus, there is a need in the art for a suitable means for an operator, such as a physician, to prepare consistent reagents without having to waste time and without having to maintain a large stock of chemicals. The means must also enable the physician to tell quickly and easily one reagent from another.

In accordance with the present invention, there is provided a disposable reagent cartridge comprising a body with at least one depression therein; and a removable cover sealed over said depression; wherein at least a reagent (which may be the same or different) is provided within each of said at least one depression and said removable cover is provided with a bar-code on an outer side thereof, said bar-code being usable to identify said reagent(s) and/or a diagnostic test requiring said reagent(s).

As mentioned above the present invention may be used for the monitoring and the diagnosis of acute myocardial infarction. Accordingly, the present invention provides a disposable reagent cartridge for diagnostic testing of myocardial infarction, the cartridge comprising a plastic body with four depressions therein and a removable cover sealed over said depressions; wherein a first depression is filled with a buffer solution, a second depression is filled with a wash solution, a third depression is filled

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with dried naphthyl phosphate, a fourth depression is filled with dried enzyme substrate (which may be alkaline phosphatase) (preferably associated with an antibody, more preferably an antibody for an antigen associated with a clinical condition - such as acute myocardial infarction) and said removable cover is printed with a bar-code on an outer side thereof, said bar-code being usable to identify said contents within one or more of the depressions (reagents) and/or the diagnostic test.

In accordance with the present invention, there is also provided a prepacked disposable diagnostic testing kit sealed with a removable cover, the kit comprising at least one disposable sample holding means, at least one disposable electrochemical biosensor, at least one disposable through-flow producing means and at least one disposable reagent cartridge, wherein said each of said at least one disposable reagent cartridge is prepacked with at least one reagent for the performance of at least one diagnostic test and then sealed with a removable seal.

In order to perform a diagnostic test, the operator (e.g. physician) need only tear off a removable cover from the kit and operate the contents thereof to perform the test. As the test may be performed with only the contents of the kit, the operator does not have to waste time cleaning any other pieces of equipment.

Embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings, in which like numerals represent like parts, and in which:

Figure 1 is a schematic representation of an automatic diagnostic apparatus;

Figure 2 is a schematic representation of a syringe and biosensor system as shown in Figure 1;

Figure 3 is a schematic representation of a rack and platform system also as shown in Figure 1;

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Figure 4 is a flow diagram generally illustrating the operation of the apparatus depicted in Figures 1, 2 and 3 under control of a controller;

Figure 5 is a schematic representation in cross-section of a container;

Figure 6 is an elevation of a reagent cartridge;

Figure 7 is a plan view of the reagent cartridge of Figure 6;

Figure 8 is a schematic representation in cross-section of an electrochemical biosensor;

Figure 9 is a plan view of a disposable diagnostic kit;

Figure 10 is a graph;

Figure 11 is a series of electrophoretograms and is taken from Figure 5 of "A Study on the Dimeric Structure of Creatine Kinase" by R.A.Wevers, H.P.Olthuis, J.C.C. van Niel, M.G.M van Wilgenburg and J.B.J. Soons, published in Clinica Chimica Acta, 75 (1977) pp 377-385;

Figure 12 is a graph and is taken from Figure 6 of "Two-Site Monoclonal Antibody Assays for Human Heart- and Brain- Type Creatine Kinase" by A.P.Jackson, K.Siddle and R.J.Thompson, published in Clinical Chemistry, Vol.30 No.7 (1984), pp 1157-1162: and

Figure 13 presents two graphs and is taken from Figure 1 of "Acute Myocardial Infarction and Coronary Reperfusion" by F.S.Apple, published in Clinical Chemistry (Review Article), A.J.C.P. February 1992 Volume 92, No.2.

Figures 14 and 15 are graphs.

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Figure 1 shows a schematic representation of an automatic diagnostic apparatus 1. The apparatus 1 comprises a controller 3 for controlling operation of the apparatus and all of the components thereof. The apparatus 3 is powered from a power supply unit 5 which includes a transformer 7. A user input 9, in this case a 16-key keypad, enables a user to input instructions and data to the controller 3. Data and instructions for the user are displayed on a display 11. Also provided for the input of data into the controller 3 is a bar code scanner 13.

The controller is connected by ribbon cables 16 to a syringe and biosensor system 15 and a rack and platform system 17. It is these systems that manipulate samples taken from a patient and generate readings therefrom.

Also provided for the output of data to a user are an RS232 port 19 and a printer interface 21 which is in turn connected to a printer 23. The RS232 port 19 may be connected to a Personal Computer (PC) if desired.

The controller is also connected to a lid sensor 25 which senses whether the apparatus's lid is open or closed. The controller will not allow the apparatus to operate until the lid of the apparatus has been closed.

Figure 2 is a schematic representation of a syringe and biosensor system 15 as shown in Figure 1. As shown, the system 15 comprises three sets of syringes 27 and associated biosensors 29. It will be appreciated, of course, that the number of sets may be varied at will. In one example, the system may be used as a means for diagnosing myocardial infarction by variations in three parameters. Tests for alternative ailments may require a fewer or greater number of sets.

The biosensors 29 are electrochemical immunoassay biosensors, and may be constructed from plastic material at a reduced unit cost. The reduced cost of these biosensors 29 enables them to be disposed of after each test without prohibitively increasing the cost of operating the apparatus. The construction of an example of the biosensor will be described later in conjunction with Figure 8. Conventional

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electrochemical immunoassay biosensors could, of course, alternatively be provided.

The syringes 27 are, in this embodiment, simple commonplace syringes which comprise a plunger 31 and a syringe body 33, and are used to generate a fluid flow through the biosensors 29. It will be understood, that whilst syringes have been described, other flow-flow producing means may alternatively be provided. For example, a fluid flow could conceivably be generated by drawing fluid through the biosensors with a pump. The pump could be connected to each of the biosensors by a disposable pipe, for example, which could be discarded after a test has been conducted.

As shown in Figure 2, one end of the plunger 31 is connected to an arm 35 of a biosensor motor 37. During use of the apparatus, the motor 37 may be operated by a biosensor motor control board 39 to move the arm 35 and attached plunger 31 in and out of the syringe body 33 thereby to generate a flow through a biosensor 29 attached to an opposite apertured end of the syringe body 33. The biosensor motor control board 39 is in turn controlled by the controller 3. Three syringe sensors 41 are provided that enable the controller 3 to sense whether a syringe 27 and attached biosensor 29 has been correctly placed in the apparatus before the testing is commenced.

A biosensor control board 43 under control of the controller 3 is provided. The board 43 is provided with contacts 45 for each biosensor 29 of the apparatus and is operable under instruction of the controller 3 to apply a voltage to each biosensor 29 as required. The biosensor control board 43 measures a current flowing through each biosensor 29, digitises the data and outputs it to the controller 3. In common with other through-flow immunoassay biosensors, the current through the biosensor is indicative of the quantity of material-to-be-sensed in a sample under test. In this embodiment, the controller 3 is an EPROM microcontroller with a 32KB (kilobyte) ROM (Read Only Memory) and a 32KB (kilobyte) RAM (Random Access Memory), although other arrangements are conceivable.

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Figure 3 is a schematic representation of a rack and platform system 17 also as shown in Figure 1. The rack and platform system comprises a block 47 with three shaped apertures 49, each for securely holding a reagent cartridge (not shown) A suitable reagent cartridge will be later described in relation to Figures 6 and 7. The block also includes an electrical heater 51 which may be used as required to heat the cartridges in the rack and platform system 17. The block 47 is provided with a heat sensor 53 which relays temperature data to the controller 3, which responds by switching on or switching off the heater 51 as required.

Whilst the apparatus of Figure 3 illustrates three apertures for holding three cartridges, it will be appreciated that a greater or lesser number of apertures and cartridges may alternatively be provided. In each of the apertures 49, a cartridge sensor 55, under control of the controller 3, is provided that senses whether a cartridge has been correctly placed in the aperture 49. If a cartridge is missing from one of the apertures 49, the controller 3 senses the absence of that cartridge and will not generate any data for the sensing system associated with that cartridge position.

Also provided is a rotor motor 57 which is operable to spin a sample container (not shown) placed in operable communication therewith. A suitable sample container is later described in relation to Figure 5. The rotor motor 57 is under the control of a motor control board 59 which is in turn controlled by the controller 3. The rack and platform system 17 is provided with a rotor sensor 61 which senses whether a sample container has been correctly placed in communication with the rotor motor 57 and communicates this information to the controller 3. The motor control board 59 also controls a rotor index motor 63 which is operable to align the rotor motor 57 and attached sample container with each sensing system of the apparatus.

The rack and platform system 17 is also provided with an up/down motor 65 and a forward/back motor 67 for moving the rack and platform system 17 in any of the aforementioned directions. The up/down and forward/back motors are controlled by the motor control board 59 in the rack and platform system 17. A pair of home sensors 69 are provided which sense when the block 47 is at it's "home" position in

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the forward/back and/or up/down directions. The "home" position is when the block 47 is at its furthest point from the sensing system in a forward/back and up/down direction. The home sensors 69 communicate position data to the controller 3.

At this juncture, it is appropriate to provide a brief general description of the manner in which the apparatus operates and is operated. Typically, a user decides, as a first step, which test they wish to perform for a particular patient. An appropriate diagnostic kit is selected and the various components removed therefrom. Next, a bar-code on the reagent cartridge (or any other part of the kit) is read with the bar-code scanner 13 and the cartridge is placed in the block aperture 49. In accordance with the bar-code, the controller 3 displays on the display 11 the type of test to be conducted and sets up the apparatus *vis-a-vis* the number of reagent compartments required and the testing routine to be undertaken. The user may then visually inspect the display 11 to check that they are indeed about to conduct the desired test.

Next, the user takes a fluid sample from a patient and places the sample in a container provided in the kit. The container is then placed in operable communication with the rotor motor 57 in the rack and platform system 17. The rack and platform system 17 is, at this stage, at its "home" position - i.e. at its furthest position from the sensing system 15 - so as to improve user accessibility to the apparatus.

It will be apparent that bar-codes may also be provided on any of the biosensor, container and syringe.

Next, the user takes a biosensor 29 and a syringe 27 from the kit, and fits them together (alternatively, the biosensor and syringe may be supplied pre-fitted together). The connected biosensor 29 and syringe 27 are then placed in the sensing system 15 with one end of the syringe's plunger 31 in communication with the biosensor system motor arm 35. The other end of the plunger 31 internally abuts the syringe's base. The biosensor 29 is fitted within the sensing system 15 in such a manner that the sensing system contacts 45 electrically connect with electrodes in the

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biosensor 29. The apparatus is now primed and ready for testing the sample.

The controller 3, via the various sensors, senses that the container, cartridge, biosensor and syringe have been correctly placed in the apparatus and waits until the closing of the apparatus lid has been sensed by the lid sensor 25. When the lid has been closed the controller 3 begins the testing process.

Firstly, the controller 3 instructs the rack and platform system motor control board 59 to operate the forward/back motor 67 so that the block 47 is withdrawn into the apparatus in such a fashion that each cartridge container is positioned below each biosensor 29.

Next, the controller 3 instructs the rack and platform system motor control board 59 to operate the rotor motor 57 and so to spin the container placed in communication therewith. The centrifuging of the sample in the container continues at approximately 400 revolutions per minute for some four minutes until the sample is properly separated (other rotational speeds may be adopted if desired). Whilst the sample is being spun, the controller 3 instructs the rack and platform system motor control board 59 to move the block 47 towards the biosensor 29 until the tip of the biosensor protrudes into a compartment of the reagent cartridge.

If the reagents need to be made up from constituents in the reagent cartridge, the controller 3 may then instruct the sensing system motor control board 39 to operate the biosensor motor 37 to move the attached syringe plunger 31 in and out of the syringe body 33 thereby to draw fluid into and to expel fluid from the biosensor 29. In addition, the controller 3 may simultaneously instruct the rack and platform system motor control board 59 to move the block 47 and hence the reagent cartridge up, down, forward or back so that reagents may be mixed between compartments of the reagent cartridge until a final desired reagent is achieved.

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Optionally, the controller 3 may then instruct the biosensor motor 37 to withdraw the plunger 31 from the syringe body 33 and draw an amount of reagent provided in the reagent cartridge through the biosensor 29. Simultaneously, the controller 3 may instruct the biosensor control board 43 to apply a voltage to the biosensor 29 and measure the current flowing in the biosensor 29. If the current is below a predetermined threshold, the controller 3 determines that the integrity of the reagent has been maintained. If, however, the current is above the threshold, then the controller determines that the integrity of the reagent has been compromised and the apparatus is halted and a suitable message displayed to the user requesting the user to replace the reagent cartridge with another reagent cartridge. The example later described below will exhibit such a step.

Next, the controller 3 instructs the rack and platform motor control board 59 to move the sample container so that the container 3 is directly below the biosensor 29. The controller 3 then instructs the rack and platform motor control board 59 to move the container so that the biosensor 29 dips into a lighter portion of the separated sample. The controller 3 then instructs the biosensor motor 37 via the biosensor motor control board 39 to move the plunger 31 and draw a quantity of separated sample into the biosensor 29. The controller 3 then instructs the rack and platform motor control board 59 to cause the movement of the cartridge until the cartridge is directly below the biosensor 29 and the biosensor 29 dips into the reagent in the reagent cartridge. The controller 3 then instructs the biosensor motor control board 39 to move the plunger 31 and draw a quantity of reagent (which may be rehydrated substrate) through the biosensor 29. As an additional step, the controller 3 may then instruct the rack and platform motor control board 39 to cause the cartridge to be moved again so that the biosensor 29 once more dips into the cartridge and a wash solution is drawn through the biosensor 29 to wash any excess reagent from the biosensor 29.

Then the controller 3 instructs the biosensor control board 43 to apply a voltage to the biosensor 29 and to measure the produced current. The current value is then communicated to the controller 3 as testing data via the ribbon cable 16.

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The controller 3 then processes the testing data and outputs the processed data to the user. The controller may also store the data so that a plurality of results may be stored over time for a particular patient. The results may then be outputted to the user in the form of a graph via the printer 23.

One example of data collection and processing will now be described. The current flowing through the sensor is recorded at precise intervals. The typical current response after applying the potential to the sensor is a decay curve. When the substrate reaches the sensor the decay quickly becomes an exponential growth curve to a peak plateau. Typically, a quantity of electrical charge is estimated initially, which is taken as the area between the two curves the lower curve being interpolated beneath the growth curve by examination of the decay rate. The turning point where decay becomes growth is called the start of peak and is determined by software in the controller by looking for a trend when the average rate of change over a number of samples reaches a threshold value. The assay result required is the concentration of analyte which is obtained by the formula:

$$\text{conc} = \frac{\text{charge} - b}{a}$$

where a and b are parameters read from the bar code or database.

Figure 4 is a flow diagram generally illustrating the operation of the apparatus depicted in Figures 1, 2 and 3 under control of a controller. With reference to Figure 4, the stages undertaken by the apparatus are as follows.

In a first step 71, the controller 3 waits for the input of bar-code information or the input of keypad information regarding the test to be undertaken. In a second step 73, the controller 3 uses the connected sensors to sense whether the container, cartridge, syringe and biosensor have been correctly placed in the apparatus. If so, then in a third step 75, the controller 3 uses the lid sensor 25 to sense whether the lid is open or closed. If the lid is closed, then the controller, in a fourth step 77, causes the spinning of the sample in the container. The controller 3, in a fifth step 79, then

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prepares the reagent(s) in accordance with the inputted bar-code or keypad information. In a sixth step 81, the controller 3 instructs the apparatus to draw separated sample through the biosensor 29 and then, in a seventh step 83, instructs the apparatus to draw the reagent(s) through the biosensor 29. In an eighth step 85, the controller 3 instructs the apparatus to apply a voltage to the biosensor 29 and, in a ninth step 87, to measure the current flowing in the biosensor 29. In a tenth step 89, the sensed current data is digitised and outputted to the controller 3 for processing in an eleventh step 91. In a final twelfth step 93, the processed data is outputted to the user.

As mentioned above, the apparatus may be used to diagnose myocardial infarction by testing three blood parameters. In such an example, the reagent cartridge would contain the following reagents in four separate compartments. The first, largest compartment would contain a buffer solution. The second compartment, smaller than the first compartment, would contain a wash solution. The third compartment, smaller than the second compartment, would contain a dried substrate (which in one example may be naphthyl phosphate). The fourth compartment, smaller than the second compartment, would contain a conjugate (which in one example may be the enzyme Alkaline Phosphatase (ALP), preferably associated with an antibody, more preferably an antibody for an antigen associated with a clinical condition - such as acute myocardial infarction).

When using such a cartridge, the buffer solution would be used to rehydrate the dried substrate and the integrity of the substrate would then be checked by way of the biosensor 29 in the above described manner. The wash solution would be used to remove any excess conjugate from the biosensor 29. In this example, the controller 3 would instruct the apparatus to perform the above mentioned additional step of testing the integrity of the rehydrated substrate by passing rehydrated substrate through the biosensor 29 whilst applying a voltage thereto. If the detected current is less than substantially 80 nA (nanoamperes), the controller 3 determines that the substrate integrity is maintained. A current level above this threshold causes the controller 3 to determine that the substrate integrity has been compromised.

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The biological processes being undertaken in the biosensor have already been described in United Kingdom Patent Publication No. 2 289 339 mentioned above, and so will not be described in any great detail herein. However, to further illuminate the operation of the present invention, a brief summary will now be given.

Figure 5 is a schematic representation in cross-section of a container 100. The container 100 comprises a substantially frusto-conical outer wall 101, with a lip 103 at its narrow end. The outer wall 101 connects at its broader end with a substantially planar annular first base 105. A second substantially conical inner wall 107 connects at its broader end with an inner edge of the annular first base 105. The inner wall 107 connects at its narrow end with a depression 109. The annular first base 105 is provided with a lip 111 on its outer edge to enable better communication of the rotor motor 57 with the container 100.

Prior to use of the apparatus, a sample of patient fluid is placed within the container 100 and the container is placed in communication with the rotor motor 57. Operation of the rotor motor 57 causes the container 100 to be spun about a central axis of the outer wall 101. Spinning of the container 100 causes heavier components of patient fluid to move towards the first base 105 and lighter components to move up the inner wall 107 to the depression 109. The lighter components are then contained within the depression 109 for facilitated removal thereof.

It will be apparent that the external configuration of the above mentioned container 100 is not essential for the function which the container 100 is to perform, namely the separation of fluid components. It is the provision of a raised depression 109 that eases the separation of fluid components when centrifuged. Thus, the container herein described is not to be read as being limited by its external configuration or shape.

Figure 6 is an elevation of a reagent cartridge 200. As shown, the reagent cartridge comprises a substantially planar body 201 with four reagent compartments (203,205,207,209) depending therefrom. The reagent compartments are open at the

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surface of the planar body 201. At one end of the cartridge, there is provided a tube 211 sized so as to accept an inlet of a biosensor therein. In this way, the reagent cartridge and the biosensor may be fitted together so that they occupy a smaller volume when packaged prior to use.

Figure 7 illustrates a top plan view of the cartridge depicted in Figure 6. As shown, the four reagent compartments are open at the planar body 201 and increase in volume from a smallest compartment 203 to a largest compartment 209. Of course, the size of the compartments may be varied at will. One end of the tube 211 is also shown in Figure 7. The first compartment 203 has an approximately circular cross section and the second 205, third 207 and fourth 209 compartments have substantially elliptical cross-sections of increasing focal spacing.

The cartridge 200 of figures 6 and 7 is initially filled with reagents for a particular diagnostic test that is to be undertaken. An example of a set of reagents for the testing of myocardial infarction (see earlier and later discussions). Once the compartments have been filled with reagent, then the cartridge 200 is sealed. Sealing of the cartridge 200 may be accomplished by adhering a removable metal foil cover to the planar body 201.

The cartridge 200 may thus be sealed and transported with a reduced risk of reagents becoming contaminated with each other, and with a reduced risk of reagents becoming spoiled. Immediately prior to use, the user can remove the cover to reveal the compartments and reagents. Alternatively, the reagent cartridge cover may be left in place and the biosensor tip may be arranged to pierce the cover where appropriate prior to removal of the cartridge contents. In either case, the user is provided with a set of reagents for a particular test without having to waste time preparing those reagents.

The cover (not shown) of the cartridge 200 may be provided with a bar-code. The bar-code gives information regarding the reagents contained within the cartridge 200 and may give information regarding the type of testing to be conducted with that

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cartridge 200.

As mentioned above, the apparatus of the present invention may be used for the diagnosis of myocardial infarction by electrochemical immunoassay. In this case, the cartridge 200 of Figure 6 and Figure 7 could be provided with the following reagents, for example. The first compartment 203 would be filled with a conjugate (which may be the enzyme ALP), the second compartment 205 would be filled with a dried substrate (which may be naphthyl phosphate), the third compartment 207 would be filled with a wash solution and the fourth compartment 209 would be filled with a buffer solution. In use, buffer solution would be taken from the fourth compartment 209 and added to the dried substrate to reconstitute the substrate solution. Other enzyme-substrate pairs are mentioned below.

Figure 8 is a schematic representation in cross-section of an electrochemical biosensor. With reference to Figure 8, the biosensor comprises a counter electrode 301, a working electrode contact 303, a biosensor body 305, a biosensor inlet 307 and a solid phase immunoassay site comprising a porous spacer disk 309, a porous PVDF disk 311 and a porous graphite disk 313 as a working electrode. The spacer disk may be a Loprosorb™ disk, for example, and the graphite disk may be a Toray™ disk (Toray Industries, Japan).

As mentioned above, the biosensor may be used for conducting an immunoassay by testing parameters of a patient's blood sample. In an example of such a test, plasma is first separated from the patient sample - preferably by use of the container of the present invention - and then drawn into the biosensor by way of a syringe attached to the counter electrode 301. As the plasma passes from the biosensor inlet 307 through the biosensor, it traverses the porous PVDF disk 311. The porous PVDF disk 311 is impregnated with a particular antibody and the drawing of plasma through the disk causes the capture of an antigen under test on the disk 311.

Next, the syringe is used to draw a quantity of tracer antibody (preferably an antibody for an antigen associated with a clinical condition - such as acute myocardial

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infarction) conjugated to alkaline phosphatase (ALP) through the biosensor. As the conjugate passes through the PVDF disk 311, the antibody marks the antigen captured on the disk 311.

Next, the syringe draws up a quantity of wash solution which is used to wash any excess conjugate from the biosensor. Next, the syringe draws up a quantity of rehydrated substrate and a potential difference is then applied to the counter and working electrodes 301, 313 and a current is produced that is indicative of the quantity of antigen captured on the disk 311.

This process functions due to the electrochemical nature of the ALP and substrate. As the ALP marks the antigen captured on the disk 311, the substrate (naphthyl phosphate) is converted to naphthol which is drawn through the biosensor 29 and oxidised on the porous graphite disk 313 by the potential difference applied thereto by the working electrode contact 303. Oxidation of the naphthol on the graphite disk 313 causes a flow of electrons (ie a current flowing in an electrical circuit comprising the counter electrode 301, aqueous solution, the working electrode 313, the working electrode contact 303 and connected devices) between the working electrode contact 303 and the counter electrode 301, the magnitude of the produced current being indicative of the quantity of naphthol oxidised at the graphite disk 313 and hence indicative of the quantity of antigen under test in the patient sample.

Whilst the above has been described in relation to an ALP enzyme and naphthyl phosphate pair, it will be understood that any enzyme-substrate combination may be used that produces a readily oxidisable or reducible species. For example, aminophenyl phosphate could be used as a substrate with ALP. Other examples of enzyme-substrate pairs are beta-galactosidase with p-Aminophenyl-beta-D-galactosidase to produce the electroactive species aminophenol, glucose oxidase with glucose to produce the electroactive species hydrogen peroxide and lactate dehydrogenase with lactate in the presence of NAD^+ to produce the electroactive species NADH.

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Figure 9 is a plan view of a disposable diagnostic kit 400. The kit 400 is particularly suitable for use with the apparatus of Figure 1. As shown in figure 9 the kit 400 comprises a container 401 within which there is provided a disposable sample container 100, a disposable syringe 27, a disposable biosensor 29 and a disposable reagent cartridge 200. The kit container 401 is provided with a removable sealed cover (not shown) which allows the sterility of the components to be maintained up to their point of use. As mentioned above, the kit 400 and its components may be manufactured at a relatively low cost from plastic material.

One highly preferred embodiment of the apparatus according to the invention will now be described. The temperature controlled block which holds the reagent strips and acts as a support for the centrifuge mechanism may be made from aluminium.

Each cartridge sensor may be a reflective optical device connected to the controller for indicating the presence of a cartridge to the microcontroller. The entire block is lifted by an up/down motor to enable sample or reagent to be drawn from the container or cartridge as required. This motor is mounted onto the a base of the apparatus.

The centrifuge is mounted on a sliding mechanism and positioned under each sensor by an index motor. The centrifuge consists of a holder into which the container is placed by the user and a guard ring to contain the container. A light sensing device is placed under the holder and interfaced to the controller to detect the presence of the sample (e.g. blood) filled container through light level changes.

The cartridges and container are positioned by a motor in a front to back direction. Since the sensor tip is fixed all samples are presented to the tip by the combination of motions of the forward/back motor, index motor and up/down motor.

The sensor system has a motor for each biosensor which drives the syringe piston through a direct linkage, in either direction as required by the controller. The lower part of the drive assembly holds the biosensor in a fixed position and provides a

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guard for the electrical contacts to the biosensor. An LED indicator is positioned adjacent each biosensor to inform the user of that biosensor's status. The electrical contacts are mounted directly onto a signal processing board which interfaces with the controller and provides a voltage to the biosensors during an assay.

The apparatus is operated by selecting pre-programmed options presented by menus which appear on the display. Bar-codes on the syringe and cartridge also provide a means of selecting test type, batch or kit calibration data etc.. The user is required to confirm the selection by keypad. A printer provides a hard copy of the result in either a text or graphical format. Should an error occur a single red LED lights and an audible alarm beeps while an error message is displayed.

A CCD (charge coupled device) type scanner reads information from bar-codes on the kit components such as the biosensor/syringe and cartridge.

The kit also has a bar-code label for entering other data. Should the label be unreadable then data is entered manually through the keypad.

Data, sensor and control signal inputs are read by the controller and processed to determine the control and data output response. The apparatus is based upon a microcontroled integrated circuit which requires external data and program memory with extra I/O (input/output) capability. The data memory is non-volatile RAM (NVR) so patient identity and results are preserved as a database when the apparatus is shut down. A real time clock is resident within the NVR to provide date and time reference during testing. The various positioning and syringe drive motors are enabled and stepped by the controller via motor drive interfaces on the motor drive boards. Biosensor power and data conversion is carried out by the biosensor signal board under microcontroller supervision. Data for printout are sent to a printer interface board and which manages the printer operation. Block temperature is controlled by the microcontroller via the block heater which contains a temperature sensor and heater power control.

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The apparatus and its components and signal elaboration software operate from mains power supplies via an IEC type inlet. The entire works and kit components are enclosed during the assay to prevent tampering.

The biosensor contains a porous disk which is impregnated with an assay specific material. Another disk in the biosensor (preferably a graphite disk) is in contact with conductive plastic parts which provide a path for current applied by the instrument during the test. Test kit reagents and sample are successively drawn through the cell by the action of the syringe piston. The speed of piston movement determines the flow rate which is controlled precisely by the controller. An air pocket inside the syringe damps drive movement to produce a smooth liquid flow through the cell. A potential is applied across the cell and the current flow measured. Analysis of this current gives the result of the assay. A bar code label is placed on the syringe to identify the assay, calibration data, batch/lot data and expiry.

The container is filled with sufficient sample (e.g. blood) to guarantee sufficient sample for three assays. High speeds are employed to produce a packed cell consistency to the haematocrit leaving plasma to be sampled. The shape of the centre of the container allows plasma to flow to the centre of the container for retention while keeping haematocrit in the outer region.

The reagent cartridge contains four compartments which hold the reagent for the assay. The reagent is sealed into the strip by a foil membrane which is pierced by the sensor tip during the assay. A bar-code is put on the strip to identify the type of assay and lot number.

<u>Use of the Apparatus to assess Acute Myocardial Infarction (AMI)</u>

The cardiac marker proteins are proteins highly specific to myocardial tissue which are released into serum during AMI tissue damage. Some of these, such as CK-MB and Myoglobin, have now been clinically validated by many studies as specific and sensitive markers for AMI. Others e.g. Troponin are growing in popularity and there

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are many groups involved in trying to discover earlier and more sensitive markers.

Table 1 (below) summarises the most popular of the markers currently available and their main characteristics. Each of these markers has something slightly different to offer in diagnosis and therapy. Myoglobin with a molecular weight of 17,000 daltons is one of the first to appear in serum or plasma after the AMI event. However it returns to normal levels within 24 hours so is not useful in diagnosing a patient who has presented some time after the symptoms commenced but would help in the decision to start thrombolytic therapy for a patient who presents early.

TABLE 1

AMI ANALYTE PANEL				
Enzyme	Rise (h)	Peak (h)	Return to Normal (h)	Notes
CK	4-6	24	48	Indicator of reperfusion
CK-MB	4-6	24	48	Specific for myocardium Indicator of reperfusion
Myoglobin	1-3	4-8	24	Very rapid
Cardiac Myosin Light Chains (cMLC)	2	high levels stable for several days	240	Related to infarct size Elevated in Unstable Angina
Troponin (T and I)	4-6	48-72	240	Highly specific for myocardium Indicator of reperfusion

To select the ideal parameters for a particular patient it is necessary to consider the general time course of these proteins in blood and their other characteristics. Figure 10 shows typical behaviour with time of these markers in a patient's serum. In this regard Figure 10 shows the concentration variation in serum with time after AMI for currently popular cardiac markers (see also Figure 14 and Figure 15).

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The apparatus of the present invention will offer the possibility to log and to present the parameters in this graphical format which allows the clinician to closely follow the patient therapy, to monitor for second infarction and to detect successful reperfusion.

In a preferred embodiment, the first panel of instrument will have the parameters Myoglobin and CK-MB in the now acceptable (and increasingly preferred) mass format (μg\L). Many clinicians would traditionally request a total CK test as well as the CK-MB Comparing CK-MB ratio to CK (when both are U\L) is a recommended criterion of the World Health Organisation (AMI if CK-MB\ CK > 4%).

The apparatus of the present invention provides a means of determining total CK as an activity measurement or an estimate of total CK. In this regard, the total CK content of serum is largely composed of the isoforms CK-MM and CK-MB and the brain enzyme CK-BB is not present in significant quantities unless there is severe head injury For example see Figure 11 which is an electrophoresis separation to illustrate CK isoforms in serum and brain extract. In these CK electrophoretograms, a = total brain extract: b = serum sample from a patient with an infarction: c = extract from the cortex of the brain: d = extract from the medulla of the brain: e = extract from the cerebellum (agarose electrophoresis 50 mM sodium barbital buffer (pH 8.0). 85 V). Thus in effect the measurement of CK-BB is not effective during AMI. A graph of CK-MB levels and CK-BB levels against time in the serum of a patient during AMI in Figure 12 also illustrates this. In this regard, Figure 12 is an illustration of CK-MB and CK-BB levels measured by two site immunoassay over time in serum from a patient suffering from AMI.

In particular Figure 12 shows a typical curve showing increase in serum CK-MB with time after myocardial infarction. As can be seen, both CK-MM and CK-MB elevate during AMI although the proportion of CK-MB to MM rises due to the high amounts of CK-MB in heart tissue. CK-MM however can also be elevated after muscle trauma as can CK-MB to a lesser extent. In practice measurement of CK-MM + CK-MB will effectively give the total CK in serum. Normally total CK is measured

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by clinical chemistry.

There are also been studies of the various isoforms in serum and how they change with time. Three types of MM exist - namely MM1, MM2 and MM3 - and two types of MB exist - namely MB1 and MB2. These are normally quantified by high voltage electrophoresis and fluorescent staining but some immunoassays are becoming available. The ratios of the MB1\MB2 and MM1\MM3 also help in the early diagnosis of AMI but some studies claim that total CK-MB measurement is just as effective. There seem to be no studies of the total CK-MB\CK-MM ratio. However, the apparatus of the present invention would be capable of performing such a study and which in theory would be very specific for AMI (setting a threshold ratio for positive diagnosis).

For the preferred apparatus and cartridge of the present invention the most convenient method will be to supply tests for myoglobin, CK-MB and CK-MM all as mass assays (via two-site immunoassay). It is quite possible that the users will use only CK-MB and Myoglobin for the majority of the patients but if they require total CK they have the option of loading to load both CK-MB and CK-MM, cartridges in one run. The instrument will give back values for CK-MM, CK-MB and estimate total CK and the CK-MB \ total CK ratio. Alternatively CK-MM can be measured on its own by the instrument.

Myoglobin remains the parameter of choice for early diagnosis of AMI - increasing in the first 1-3 hours after AMI, peaking around 6 hours after and returning to normal within 24 hours. The current threshold for AMI with Myoglobin is > 90 μg\L although this could be clinically verified using the apparatus of the present invention.

CK-MB threshold levels for AMI have been set at around 5 μg\L in other manufacturer's kits.

Both CK-MB and Myoglobin can be used to monitor reperfusion. Figure 13 shows the difference between reperfused and non-reperfused CK-MB levels in two patients

after rt-PA therapy.

In this regard, Figure 13 illustrates CK-MB measurement with time in reperfused patients and non-reperfused patients, wherein serial total CK (left) and CK-MB (right) values for two patients following myocardial infarction: one successfully reperfused after recombinant tissue-type plasminogen activator (rt-PA) therapy (reperfusion): one not reperfused.

In summary, therefore, the biosensor system of the present invention allows sensitive immunoassays to be performed in less than 15 minutes in the ward or satellite laboratory. The present invention is particularly of use in the areas of emergency cardiology, critical care units and other departments concerned with the diagnosis and treatment of acute myocardial infarction (AMI). In a preferred embodiment, the system is capable of performing up to three immunoassay parameters simultaneously on one patient sample in less than fifteen minutes. In the cardiology sector the instrument will act as a diagnostic aid for AMI and as a means of monitoring reperfusion. In a preferred embodiment, the three parameters offered on the first panel will be myoglobin, CK-MB and CK-MM (for total CK).

The instrument of the present invention can be small and light, and can be easily carried around a ward to different locations or suitable for transportation on a small trolley. Typically, an operator will load 3 mls of heparinised blood from the patient into a disposable plastic rotor which is then placed in the machine. For each parameter there is a small syringe and reagent cartridge which will be packaged together and bar coded for a specific test (myoglobin, CK-MB etc.). The operator uses a wand - type bar code reader to swipe the details from the side of the syringe and the machine lights up an LED where the syringe is to be loaded and checks on the display that the operator wants to test this parameter for the current patient sample. This is repeated for the cartridge. One, two or three parameters can be run for any patient sample in one cycle of the machine.

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When the lid of the instrument is closed the apparatus goes into its routine. Typically, the blood is centrifuged for 4 minutes and during that time the instrument is priming and checking the electrochemical biosensors.

At the end of the period typically 250 μl of plasma is aspirated directly from the disposable rotor into each of the syringe heads. In a preferred embodiment the plasma passes through the syringe head it traverses a porous antibody-coated membrane and the antigen being tested is captured. The syringe then goes to the cartridge and typically draws up 500 μl of tracer antibody conjugated to alkaline phosphatase (ALP). This passes through the membrane marking the captured antigen.

In this preferred embodiment, the syringe next draws up wash solution (1 ml) and then goes to the enzyme substrate well on the cartridge. Inside the syringe head (behind the antibody-coated membrane) is a porous electrode with a second return electrode located further along the head. The ALP substrate used is electrochemical in that contact with ALP converts the substrate (naphthyl phosphate into an electroactive product (naphthol) which is easily oxidised on the porous electrode. The assay is calibrated for each antigen so that current at the electrode corresponds to antigen concentration.

Typically, all three parameters are completed within 15 mins and the instrument will display concentrations, print out concentrations on request and also print graphs for each parameter against time if previous values have been stored for that patient.

In a preferred embodiment, the instrument is capable of storing 24 values for each of the three parameters for up to a maximum of 15 patients.

Thus, the apparatus of the present invention uses an *in vitro* electrochemical assay technique to determine heart attacks by measuring the levels of specific markers in a patient's or victim's blood sample. The levels of markers indicate the time and severity of the attack and also the progress of recovery.

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Thus, also, the apparatus of the present invention is an instrument into which one use disposable kit components and blood sample are loaded in order to obtain a result. The kit components consist of an electrochemical cell and syringe, a reagent strip and a sample holder (otherwise known as a centrifuge rotor).

The syringe and strip are bar-coded for correct identification and assay/calibration data. Each marker requires a specific type of cell.

The apparatus of the present invention performs the assay automatically once the assay kit components have been loaded and verified by the bar-code matching and the operators confirmation. The patients blood is measured into the rotor and loaded onto the instrument at the beginning of the test. The assay is performed automatically and results are stored internally for display or printout as required.

It will be understood that the present invention has been described herein by way of example only and that modifications and additions may be made within the scope of the invention.

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CLAIMS

1. An automatic diagnostic apparatus comprising:

a controller for controlling operation of the apparatus and for processing data;

a sensing system operably connected to the controller for performing an assay, preferably an electrochemical assay (more preferably an electrochemical immunoassay), of a sample and communicating data from said assay to said controller;

voltage supply means for applying a potential difference to said sensing system; and

output means for communicating processed data to a user.

2. An apparatus according to Claim 1, further comprising sample holding means for holding said sample.

3. An apparatus according to Claim 2 wherein said sample holding means comprises a container having a first base and a second base, said second base being raised from said first base and having a depression provided therein, such that when material comprising a heavier component and a lighter component is placed within said container and spun, said heavier component is forced towards said first base and said lighter component is forced towards and onto said second base and subsequently retained within said depression.

4. An apparatus according to Claim 2 or Claim 3, wherein said apparatus further comprises a centrifuge for spinning said sample holding means.

5. An apparatus according to any one of Claims 2 to 4, wherein said sample holding means further comprises reagent holding means.

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6. An apparatus according to Claim 5, wherein said reagent holding means is a reagent cartridge comprising a body with at least one depression therein; and a removable cover sealed over said depression; wherein at least a reagent is provided within each of said at least one depression and said removable cover is provided with a bar-code on an outer side thereof, said bar-code being usable to identify said reagent(s) and/or a diagnostic test requiring said reagent(s).

7. An apparatus according to Claim 5 or Claim 6, comprising heating means for heating said reagent holding means, said heating means being controlled by said controller.

8. An apparatus according to any one of Claims 1 to 7, comprising input means for inputting data into said controller.

9. An apparatus according to Claim 8 wherein said input means comprises a keypad, and a scanner for scanning bar-code data.

10. An apparatus according to any preceding claim wherein said apparatus has a lid and wherein said controller is operably connected to a lid sensor for sensing whether the apparatus's lid is open or closed.

11. An apparatus according to any preceding claim wherein said controller is operably connected to a sample sensor for sensing whether a sample is present.

12. An apparatus according to any preceding claim wherein said sensing system comprises:

a electrochemical immunoassay biosensor for performing an electrochemical immunoassay of a sample; and

means for generating flow of said sample through said biosensor.

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13. An apparatus according to Claim 12 wherein said biosensor comprises:

a sensor body having a sensor outlet in a part thereof;

a counter electrode having a first aperture operably connected to said sensor outlet;

a working electrode having a second aperture operably connected to said sensor outlet;

a solid phase system operably located within said working electrode, and

an inlet means to provide a sample onto said solid phase system.

14. An apparatus according to Claim 13, wherein said sensor body is manufactured from a plastics material and said working and counter electrodes are manufactured from an electrically conductive plastics material.

15. An apparatus according to Claim 12 or Claim 13, wherein said biosensor is that of GB-A-2289339.

16. An apparatus according to any one of Claims 12 to 15 wherein said means for generating said flow is a syringe.

17. An apparatus substantially as hereinbefore described and as shown in the accompanying drawings.

18. Use of an apparatus according to any one of claims 1 to 17 to diagnose and monitor a clinical condition, in particular acute myocardial infarction.

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19. A method of automatic diagnosis, the method comprising the steps of:

(a) placing a sample within an automatic diagnostic apparatus;

(b) generating instructions with a controller for instructing a voltage supply means to apply a voltage to a sensing system;

(c) controlling said sensing system with said controller to perform an assay, preferably an electrochemical assay (more preferably an electrochemical immunoassay), of said sample and to generate data for output to said controller;

(d) processing said data in said controller to generate processed data; and

(e) controlling with said controller an output means to output said processed data to a user.

20. A method of automatic diagnosis according to Claim 19, conducted with an automatic diagnostic apparatus according to any one of Claims 1 to 17.

21. A disposable electrochemical immunoassay biosensor comprising:

a sensor body with a depression therein and a sensor outlet in said depression;

an apertured counter electrode provided in abutment with one side of said depression such that said counter electrode aperture communicates with said outlet;

an apertured working electrode provided in abutment with another side of said depression such that said working electrode aperture communicates with said sensor outlet;

an immunoassay system provided in close proximity to said working electrode,

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and

an apertured sensor inlet means also provided within said working electrode and in communication with said immunoassay system;

wherein said sensor body is manufactured from a plastics material and said working and counter electrodes are manufactured from an electrically conductive plastics material.

22. A disposable electrochemical immunoassay biosensor according to Claim 21 wherein said immunoassay system is within said working electrode.

23. A prepacked disposable diagnostic testing kit sealed with a removable cover, the kit comprising at least one disposable sample holding means, at least one disposable electrochemical biosensor, at least one disposable through-flow producing means and at least one disposable reagent cartridge, wherein said each of said at least one disposable reagent cartridge is prepacked with at least one reagent for the performance of at least one diagnostic test and then sealed with a removable seal.

24. A kit according to Claim 23, wherein said sample holding means comprises a container as defined in Claim 3.

25. A kit according to Claim 23 or Claim 24, wherein said electrochemical biosensor comprises a biosensor according to Claim 21 or Claim 22.

26. A kit according to any one of Claims 23 to 25, wherein said through flow producing means is a syringe.

27. A kit according to any one of Claims 23 to 26, wherein said at least one reagent cartridge is a cartridge as defined in Claim 6.

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28. A container having a first base and a second base, said second base being raised from said first base and having a depression provided therein , such that when material comprising a heavier component and a lighter component is placed within said container and spun, said heavier component is forced towards said first base and said lighter component is forced towards and onto said second base and subsequently retained within said depression.

29. A disposable reagent cartridge comprising a body with at least one depression therein; and a removable cover sealed over said depression; wherein at least one reagent is provided within said depression and said removable cover is printed with a bar-code on an outer side thereof, said bar-code being usable to identify said reagent and/or a diagnostic test requiring that reagent.

30. A reagent cartridge according to Claim 29 comprising at least one depression filled with buffer solution.

31. A reagent cartridge according to Claim 30 comprising at least one depression filled with a dried substrate that is dissolvable by mixing with said buffer solution.

32. A reagent cartridge according to Claim 31 wherein said substrate is naphthyl phosphate.

33. A reagent cartridge according to any of Claims 29 to 32 comprising at least one depression filled with a wash solution.

34. A reagent cartridge according to any of Claims 29 to 33 comprising at least one depression filled with a conjugate solution.

35. A reagent cartridge according to Claim 34 wherein said conjugate is alkaline phosphatase, preferably having associated therewith an antibody.

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36. A disposable reagent cartridge for diagnostic testing of myocardial infarction, the cartridge comprising a plastic body with four depressions therein and a removable cover sealed over said depressions; wherein a first depression is filled with a buffer solution, a second depression is filled with a wash solution, a third depression is filled with dried naphthyl phosphate, a fourth depression is filled with alkaline phosphatase, preferably associated with an antibody, and said removable cover is printed with a bar-code on an outer side thereof, said bar-code being usable to identify said contents within one or more of the depressions and/or the diagnostic test.

37. A method of automatically diagnosing myocardial infarction, the method comprising monitoring *ex vivo* levels of one or more detectable cardiac marker proteins, such as any one or more of CK, CK-MM, CK-MB, myoglobin, cardiac myosin light chain(s), Troponin T or Troponin I, or a cardiac marker suitable for the diagnosis of acute myocardial infarction.

38. A method according to Claim 36 accomplished with the apparatus according to any one of Claims 1 to 17.

39. A conducting plastic electrode suitable for use in a diagnostic apparatus.

40. Use of a conducting plastic electrode for an electrochemical immunoassay.

41. An automatic diagnostic apparatus comprising:

a controller for controlling operation of the apparatus and for processing data;

a sensing system for performing an assay of a sample, and for communicating sensed information to the controller; and

output means for communicating processed data to the user.

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42. Apparatus according to claim 42, further comprising means for supplying a power or voltage signal to the sensing system.

43. Apparatus according to claim 41 or 42, wherein the controller is operable to control at least partly the operation of the sensing system.

44. A self contained diagnostic apparatus comprising:

a centrifuge;

a system for collecting and temporarily storing material from the centrifuge after spinning;

means for transferring the collected material to or through a sensor for performing an assay on the collected material;

means for transferring one or more other materials to or through the sensor;

an electronic controller for controlling operation of the apparatus and for processing output information from the sensor.

45. Apparatus according to claim 44, further comprising means for receiving a cartridge containing said one or more other materials for the sensor, and wherein said means for transferring said one or more other materials, compress means for obtaining said materials from the cartridge and, preferably, for temporarily storing said material.

46. Apparatus according to claim 44 or 45, comprising multi-channel collecting means for handling and/or creating a plurality of samples.

47. Apparatus according to claim 44, 45 or 46 wherein the sensor is interchangeable.

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48. A method of automatic diagnosis, the method comprising the steps of:

operating a sensing system under the control of a controller to perform an assay of a sample and to generate output information to the controller;

processing said information in said controller; and

outputting information from the controller to the user.

49. A method according to claim 48, comprising the steps of applying a power or voltage signal to the sensing system under the control of the controller.

50. A carrier for carrying material in a centrifuge and having first and second regions such that, in use, during spinning in a centrifuge a heavier component of the material collects in one of the regions, and a lighter component of the material collects in the other regions, the carrier being configured to obstruct re-mixing of the component after spinning.

51. A carrier according to claim 50, wherein the carrier has a barrier wall between the first and second regions for obstructing mixing of the components.

52. A carrier according to claim 51, wherein the first region comprises a depression, a wall thereof forming the barrier wall.

53. A disposable reagent cartridge substantially as hereinbefore described with reference to Figures 6 and 7 of the accompanying drawings.

54. A container substantially as hereinbefore described with reference to Figure 5 of the accompanying drawings.

55. A biosensor substantially as hereinbefore described with reference to Figure 8 of the accompanying drawings.

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56. A kit substantially as hereinbefore described with reference to Figure 9 of the accompanying drawings.

57. A method of automatic diagnosis substantially as hereinbefore described.

58. A method of automatically diagnosing myocardial infarction substantially as hereinbefore described.

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The Patent Office

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Application No: GB 9705243.5 **Examiner:** David Mobbs
Claims searched: 1-21, 41-43, 48-49 **Date of search:** 1 May 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): G1N NBMA, NBMX.

Int Cl (Ed.6): G01N 33/483, 33/487, 33/543, 35/00.

Other: ONLINE: WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	EP 0,359,049 A	(HITACHI, LTD.)	1-2, 5, 8, 11, 19-20, 41-43, 48-49
Y	EP 0,352,689 A	(GUIGAN)	3-4.
X, Y	WO 95/02189 A	(ABAXIS, INC.)	X: 1-2, 5, 7-8, 11, 19-20, 41-43, 48-49; Y: 3-4.
X	WO 94/19684 A	(DIAMETRICS MEDICAL, INC.)	1-2, 5, 11, 18, 41, 43, 48.
X	WO 92/05449 A	(LEHNER)	1-2, 5, 8, 11, 19-20, 41-43, 48-49.
X	WO 90/01700 A	(BATTELLE MEMORIAL INSTITUTE)	1-2, 5, 8-9, 19-20, 41-43, 48-49.
X	US 4,974,592	(AMERICAN SENSOR SYSTEMS CORPORATION)	1-2, 5, 18-20, 41-43, 48-49.

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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Phone:		**Date:**	November 21, 2003
Re:	09/942,515	**CC:**	

Applicants: Bhullar et al.
Serial No.: 09/942,515
Filed: August 29, 2001
Entitled: BIOSENSOR
Ref. No.: RDID 00108 US
Examiner: Olsen, Kaj K
Group: 1753

Attachments:

- Transmittal Letter (1pp)
- Reply In Response To Restriction Requirement (5pp)
- Fax Transmittal (1pp)

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Application Number	09/942,515
Filing Date	August 29, 2001
First Named Inventor	Bhutler
Art Unit	1753
Examiner Name	Olsen, Kai K
Attorney Docket Number	RDID 00108 US

Total Number of Pages in This Submission: 7

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
- ☐ Fee Attached
- ☑ Amendment/Reply
- ☐ After Final
- ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
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Fax Transmittal (1pp)

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	The Law Office of Jill L. Woodburn, L.L.C. Jill L. Woodburn
Signature	Jill L. Woodburn
Date	November 21, 2003

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Docket No. RDID 00108US *PATENT*

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In re application of: Bhullar et al.

Application No.: 09/942,515 Group No.: 1753

Filed: August 29, 2001 Examiner: Olsen

For: BIOSENSOR

Mail Stop Non Fee Amendment
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P.O. Box 1450
Alexandria, VA 22313-1450

REPLY IN RESPONSE TO RESTRICTION REQUIREMENT

Sir:

INTRODUCTORY COMMENTS

This is submitted in response to the restriction requirement of November 17, 2003. In response thereto, Group I, i.e., Claims 1-24 and 30-32 are elected.

PAGE 3/7 * RCVD AT 11/21/2003 5:26:26 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/3 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):01-42

IN THE CLAIMS

Claim 1 (Original): A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and

at least one reagent positioned on at least one electrode.

Claim 2 (Original): The biosensor of claim 1 wherein the code pattern is a bar code.

Claim 3 (Original): The biosensor of claim 1 wherein the conductive coating is gold.

Claim 4 (Original): The biosensor of claim 1 wherein the code pattern is optically discernible.

Claim 5 (Original): The biosensor of claim 1 further comprising a second electrically conducive coating positioned on the code pattern.

Claim 6 (Original): The biosensor of claim 1 wherein the code pattern is electrically discernible.

Claim 7 (Original): The biosensor of claim 1 wherein the code pattern includes recesses formed in the conductive coating.

Claim 8 (Original): The biosensor of claim 1 wherein the support substrate includes opposite first and second ends and the code pattern is positioned adjacent to the second end.

Claim 9 (Original): The biosensor of claim 1 further comprising a cover substrate extending across at least a portion of the electrodes.

Claim 10 (Original): The biosensor of claim 1 wherein code pattern includes pads that are spaced-apart from the surrounding electrically conductive coating.

Claim 11 (Original): The biosensor of claim 10 wherein the pads are isolated from one another.

Claim 12 (Original): The biosensor of claim 10 wherein the pads are interconnected.

Claim 13 (Original): A biosensor comprising:

a support substrate,

PAGE 4/7 * RCVD AT 11/21/2003 5:26:26 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/3 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):01-42

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the code pattern is discernible, and

a cover cooperating with the support substrate to define a channel and at least a portion of the electrodes are positioned in the channel.

Claim 14 (Original): The biosensor of claim 13 wherein the code pattern is optically discernible.

Claim 15 (Original): The biosensor of claim 13 further comprising a second electrically conducive coating positioned on the code pattern.

Claim 16 (Original): The biosensor of claim 13 wherein the code pattern is electrically discernible.

Claim 17 (Original): The biosensor of claim 13 wherein the support substrate includes opposite first and second ends and the code pattern is positioned adjacent to the second end.

Claim 18 (Original): The biosensor of claim 17 wherein the electrodes cooperate to define an array positioned adjacent to the first end.

Claim 19 (Original): The biosensor of claim 18 wherein the channel extends from the first end to the array.

Claim 20 (Original): The biosensor of claim 13 wherein the code pattern is a bar code.

Claim 21 (Original): The biosensor of claim 20 wherein the conductive coating is gold.

Claim 22 (Original): The biosensor of claim 13 wherein code pattern includes pads that are spaced-apart from the surrounding electrically conductive coating.

Claim 23 (Original): The biosensor of claim 22 wherein the pads are isolated from one another.

Claim 24 (Original): The biosensor of claim 22 wherein the pads are interconnected.

Claims 25-29 (Cancelled).

Claim 30 (Original): A biosensor comprising:

a support substrate,

PAGE 5/7 * RCVD AT 11/21/2003 5:26:26 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/3 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):01-42

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and means for identifying the biosensor, wherein there is sufficient contrast between the conductive coating and the substrate such that the identifying means is discernible.

Claim 31 (Original): The biosensor of claim 30 further comprising a cover that cooperates with the support substrate to define a channel and at least a portion of the electrodes are positioned in the channel.

Claim 32 (Original): The biosensor of claim 30 wherein the identifying means is a bar code.

PAGE 6/7 * RCVD AT 11/21/2003 5:26:26 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/3 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):01-42

REMARKS

Claims 1-24 and 30-32 are pending in the application.

Claims 25-29 are cancelled without prejudice or disclaimer of the subject matter claimed therein. The right to file a divisional application directed to the cancelled claims is acknowledged.

The claims are believed to be in condition for allowance, and allowance of the application is respectfully requested. It is requested that this paper be considered a Petition for Extension of time sufficient to effect a timely response, and that all fees due be charged to Deposit Account Number 02-2958 with reference to (RDID 00108 US).

Respectfully submitted,
The Law Office of Jill L. Woodburn, L.L.C.

November 21, 2003
(Date)

Jill L. Woodburn
Registration No. 39,874

PAGE 7/7 * RCVD AT 11/21/2003 5:26:26 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-1/3 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):01-42

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US	8155

32842 7590 11/17/2003

THE LAW OFFICE OF JILL L. WOODBURN, L.L.C.
JILL L. WOODBURN
128 SHORE DR.
OGDEN DUNES, IN 46368

EXAMINER	
OLSEN, KAJ K	

ART UNIT	PAPER NUMBER
1753	

DATE MAILED: 11/17/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	09/942,515	BHULLAR ET AL.	
	Examiner	Art Unit	
	Kaj Olsen	1753	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-32* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

   I. Claims 1-24 and 30-32, drawn to biosensor, classified in class 204, subclass 403.01.

   II. Claims 25-29, drawn to method of forming biosensor, classified in class 204, subclass 298.36.

The inventions are distinct, each from the other because of the following reasons:

2. Inventions II and I are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case the product can be made by screen printing the code pattern.

3. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

4. A telephone call was made to Jill Woodburn on 11-10-2003 to request an oral election to the above restriction requirement, but did not result in an election being made.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kaj Olsen whose telephone number is (703) 305-0506. The examiner can normally be reached on Monday through Thursday from 7:00 AM-4:30 PM. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner are unsuccessful, the examiner's supervisor, Mr. Nam Nguyen, can be reached at (703) 308-3322.

When filing a fax in Group 1700, please indicate in the header "Official" for papers that are to be entered into the file, and "Unofficial" for draft documents and other communications with the PTO that are not for entry into the file of this application. This will expedite processing of your papers. The fax number for regular communications is (703) 305-3599 and the fax number form after-final communications is (703) 305-5408.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist, whose telephone number is (703) 308-0661.



Kaj K. Olsen
Patent Examiner
AU 1753
November 10, 2003

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US	8155

32842 , 7590 12/24/2003

THE LAW OFFICE OF JILL L. WOODBURN, L.L.C.
JILL L. WOODBURN
128 SHORE DR.
OGDEN DUNES, IN 46368

EXAMINER	
OLSEN, KAJ K	
ART UNIT	**PAPER NUMBER**
1753	

DATE MAILED: 12/24/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/942,515	BHULLAR ET AL.	
	Examiner	Art Unit	
	Kaj Olsen	1753	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 November 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *1/27,8/29* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 1-24 and 30-32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

3. In claims 1, 13, and 30, it is unclear what the metes and bounds of a "discernible" code pattern would be. Discernible by what and discernible for what purpose? The full scope of what could reasonably be construed as being a "discernable code" by one possessing ordinary skill in the art is unclear.

4. Claim 13 is further indefinite because it is unclear what a "sufficient contrast" would be when the applicant hasn't specified what kind of discernment is being utilized.

5. Claim 30 is further indefinite because applicant first sets forth the presence of a "mean for identifying" and later refers to it as being an "identifying means". Although the examiner can recognize that the applicant is referring to the same thing in each case, this is potentially confusing because means-plus-function language can be interpreted with different scope than function-means language. Applicant should be consistent in the use of the term "means" to avoid any confusion.

Specification

6. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed.

The following title is suggested: Biosensor with deposited code pattern.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

8. Claims 1, 4-19, 22-24, 30, and 31 are rejected under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Kawanaka et al (USP 6,599,406 B1).

9. Kawanaka discloses a biosensor comprising a support substrate with electrodes and a pattern of terminals that are utilized for the determination of details about the biosensor (fig. 12 and 13 and col. 17, line 51 through col. 19, line 31). Because the layout of the pattern varies depending on the type of sensor present (e.g. compare figures 12, 13, and 15), this pattern thereby reads on the applicants "discernible" pattern or the means for identifying the the biosensor giving the claim language its broadest reasonable interpretation. Kawanaka also discloses the presence of a reagent (col. 10, lines 8-21). With respect to the code pattern and electrode being defined by the same electrically conductive coating, the determination of

patentability for the claim is based on the product itself. Because the product of the claim is identical to the invention of Kawanaka the process from which it was made is the same as or obvious over the process utilized by Kawanaka (see *In re Thorpe*, 777 F.2d 695, 698). However, it appears that the code pattern is deposited as the same time as the electrodes.

10. With respect to the pattern being electrically discernible, the various terminals on the test strip are for electrically interacting with the measuring apparatus.

11. With respect to the pattern being optically discernible, because the terminals are carbon and the substrate is not, the pattern would clearly be optically discernible (i.e. the naked eye could distinguish where carbon is and is not deposited). Although the reference doesn't state the use of optical discernment, that is only the intended use of the apparatus and the intended use need not be given further due consideration in determining patentability. The pattern only need be optically discernible.

12. With respect to the recess, the gap between electrodes 5 and 6 in fig. 33 would constitute a recess in the conductive coating.

With respect to the presence of a second conductive coating, because this limitation is an extension of a process of making the coating pattern (which was not given further due consideration as set forth above) and because this limitations doesn't specify the second coating must be compositionally different from the first coating, this limitation would read on the mere use of a second coating step where the same electrode ink is deposited a second time. Again the determination of patentability for the claim is based on the product itself. Because the product of the claim is identical to the invention of Kawanaka the process from which it was made is the same as or obvious over the process utilized by Kawanaka (see *In re Thorpe*, 777 F.2d 695, 698).

13. With respect to the use of a cover, see fig. 33 and 34.

14. With respect to the code patterns being either isolated or interconnected, the various embodiments of fig. 12, 13, 15, 20, 22, 24, and 28-32 all teach combinations of both.

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

17. Claims 3 and 21 (and claims 5 and 15 in the alternative) are rejected under 35 U.S.C. 103(a) as being unpatentable over Kawanaka in view of Song et al (USP 5,567,302).

18. Kawanaka set forth all the limitations of claims 3 and 21, but did not explicitly set forth the use of gold for the conductive coating. However, the use of noble metals such as gold are well known in the art of biosensors because these metals are highly conductive, but still inert in biological fluid. In particular, Song teaches that gold or carbon (which was utilized by

Kawanaka) both find utility for electrode materials (col. 19, lines 1-16). It would have been obvious to one of ordinary skill in the art at the time the invention was being made to utilize the teaching of Song for the biosensor of Kawanaka both because the substitution of one known electrode material for another requires only routine skill in the art and because gold provides high conductivity and inertness.

19. With respect to claims 5 and 15 in the alternative, Song teaches that when gold is to be utilized as an electrode (which was obvious to utilized as suggested above), the gold should be the second conductive layer deposited (chromium should be deposited first for adhesion purposes)(col. 16, lines 30-43). Hence, it would have been obvious to one of ordinary skill in the art at the time the invention was being made to deposit two conductive layers such that the electrode layer has good adhesion to the substrate.

Allowable Subject Matter

20. Claims 2, 20, and 32 would be allowable if rewritten to overcome the rejection(s) under 35 U.S.C. 112, second paragraph, set forth in this Office action and to include all of the limitations of the base claim and any intervening claims.

21. The following is a statement of reasons for the indication of allowable subject matter: Although the prior art recognizes the inclusion of barcodes onto electrode containing biosensors (e.g. see Poppe et al references), the prior art does not disclose nor render obvious a bar code defined by the electrically conductive coating utilized to define the electrodes.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kaj Olsen whose telephone number is (703) 305-0506. The examiner can normally be reached on Monday through Thursday from 7:00 AM-4:30 PM. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner are unsuccessful, the examiner's supervisor, Mr. Nam Nguyen, can be reached at (703) 308-3322.

When filing a fax in Group 1700, please indicate in the header "Official" for papers that are to be entered into the file, and "Unofficial" for draft documents and other communications with the PTO that are not for entry into the file of this application. This will expedite processing of your papers. The fax number for regular communications is (703) 305-3599 and the fax number form after-final communications is (703) 305-5408.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist, whose telephone number is (703) 308-0661.



Kaj K. Olsen
Patent Examiner
AU 1753
December 13, 2003

U.S. Department of Commerce Patent and Trademark Office	ATTY DOCKET NO. RDID 0003 US	SERIAL NO. To Be Assigned
	APPLICANT Bhullar et al.	
INFORMATION DISCLOSURE STATEMENT	FILING DATE Herewith	GROUP To Be Assigned

JC826 U.S. PTO
09/942515
08/29/01

*Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
KO	BA	5,438,271	8/1/95	White et al.	324	444	
KO	BB	5,762,770	6/9/98	Pritchard et al.	204	403	
KO	BC	5,798,031	8/25/98	Charlton et al.	204	403	
KO	BD	5,989,917	11/23/99	McAleer et al.	436	46	
KO	BE	5,997,817	12/7/99	Crismore et al.	422	58	
KO	BF	6,036,092	3/14/00	Lappe	235	462.13	
KO	BG	6,258,229	7/01	Winarta et al.	204	403	
	BH						
	BI						
	BJ						
	BK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes No
	BL						
	BM						
	BN						
	BO						
	BP						

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, etc.)

	BR	
	BS	
	BT	

Examiner	Date Considered 12/13/03

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

U.S. Department of Commerce Patent and Trademark Office	ATTY DOCKET NO.	SERIAL NO.
	RDID 00108	To Be Assigned
	APPLICANT	
INFORMATION DISCLOSURE STATEMENT	Bhullar et al.	
	FILING DATE Herewith	GROUP To Be Assigned

JC628 U.S. PTO 09/942515 08/29/01

*Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
KO	AA	4,476,149	10/9/84	Poppe et al.	~~427~~	2	
KO	AB	4,592,893	6/3/86	Poppe et al.	~~422~~	~~56~~	
KO	AC	4,671,661	6/9/87	Ott	~~356~~	~~402~~	
KO	AD	4,963,814	10/16/90	Parks et al.	~~323~~	~~274~~	
KO	AE	4,999,582	3/12/91	Parks et al.	~~324~~	~~438~~	
KO	AF	4,999,632	3/12/91	Parks	~~324~~	~~167~~	
KO	AG	5,243,516	9/7/93	White	~~364~~	~~413.07~~	
KO	AH	5,352,351	10/4/94	White et al.	~~204~~	~~406~~	
KO	AI	5,366,609	11/22/94	White et al.	~~204~~	~~403~~	
KO	AJ	5,405,511	4/11/95	White et al.	~~204~~	~~153.1~~	
KO	AK	5,413,690	5/9/95	Kost et al.	~~204~~	~~403~~	

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes No
KO	AL	WO 99/22236	5/6/99	PCT			
KO	AM	WO 99/36901	7/22/99	PCT			
KO	AN	WO 99/39298	8/5/99	PCT			
KO	AO	WO 00/73778	12/00	PCT			
KO	AP	WO 00/73785	12/00	PCT			

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, etc.)

KO	AR	LPKF MicrolineLaser II, LPKF Laser & Electronics AG; LPKF: Art.-Nr. 107645-2 (01/00) (2pp.) date Unknown
KO	AS	Microline Solutions, LPKF Laser & Electronics AG; LPKF: Art.-Nr. 107658-1 (01/00) (4pp.) date unknown
	AT	

Examiner [signature] Date Considered 12/13/03

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

OIPE JC29 JAN 27 2003 PATENT & TRADEMARK OFFICE

U.S. Department of Commerce Patent and Trademark Office	ATTY DOCKET NO.	SERIAL NO.
	RDID 00108	09/942,515
INFORMATION DISCLOSURE STATEMENT	APPLICANT: Bhullar et al.	
	FILING DATE: 29-Aug-01	GROUP: 1743

*Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes No
KO	AL	2 311 614 A	10-01-97	GB	G~~01N27~~	~~327~~	--
	AM						
	AN						
	AO						
	AP						

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, etc.)

	AR	
	AS	
	AT	

Examiner: Kay Olan Date Considered: 12/13/03

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Notice of References Cited	Application/Control No. 09/942,515	Applicant(s)/Patent Under Reexamination BHULLAR ET AL.	
	Examiner Kaj Olsen	Art Unit 1753	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,567,302	10-1996	Song et al.	205/777.5
	B	US-6,599,406	07-2003	Kawanaka et al.	204/403.02
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application No.	Applicant(s)	
	09/942,515	BHULLAR ET AL.	
	Examiner	Art Unit	
	Kaj Olsen	1753	

SEARCHED			
Class	Subclass	Date	Examiner
204	403.01	12/12/03	KKO
↓	403.02	↓	↓
422	82.01	↓	↓

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
inventor name search	12/12/03	KKO
BRS search of USPAT database	12/12/03	KKO

Index of Claims

Application No.	Applicant(s)	
09/942,515	BHULLAR ET AL.	
Examin r	Art Unit	
Kaj Olsen	1753	

√	R j ct d	–	(Through num ral) Cancelled	N	Non-El ct d	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

Claim		Date
Final	Original	12/3/03
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12	IS&R	7160	((204/400-435) or (205/775-794.5)).CCLS.	USPAT; US-PGPUB	2003/12/12 12:24
13	BRS	51	(((204/400-435) or (205/775-794.5)).CCLS.) and (barcod$4 or (bar adj cod$4))	USPAT; US-PGPUB	2003/12/12 12:35

	Type	Hits	Search Text	DBs	Time Stamp
14	BRS	438	(sputter$7 or ablat$6 or (screen adj print$5) or deposit$5) with (barcod$4 or (bar adj cod$4))	USPAT; US-PGPUB	2003/12/12 12:37
15	BRS	161	((sputter$7 or ablat$6 or (screen adj print$5) or deposit$5) with (barcod$4 or (bar adj cod$4))) and (sensor or biosensor)	USPAT; US-PGPUB	2003/12/12 12:57
16	BRS	91	(sputter$7 or ablat$6 or (screen adj print$5)) with (barcod$4 or (bar adj cod$4))	USPAT; US-PGPUB	2003/12/12 14:27
17	BRS	23	((sputter$7 or ablat$6 or (screen adj print$5)) with (barcod$4 or (bar adj cod$4))) and (sensor or biosensor)	USPAT; US-PGPUB	2003/12/12 12:57

Atty. Docket No. RDID 00108 US *OFFICIAL*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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APR 2 0 2004

In re application of: Bhullar et al.

Application No.: 09/942,515 Group No.: 1753

Filed: 08/29/2001 Examiner: Olsen, Kaj K

For: Biosensor with Code Pattern (amended)

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

OFFICIAL

Sir:

AMENDMENT AND REPLY UNDER 37 C.F.R. 1.111

INTRODUCTORY COMMENTS

In response to the Official Action mailed December 24, 2003, request for a one-month extension of time, entry of the following amendments and consideration of the accompanying remarks is respectfully requested.

PAGE 5/14 * RCVD AT 4/20/2004 3:03:23 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/11 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):04-34

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Fax

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To:	Commissioner for Patents	**From:**	Jill Woodburn
Fax:	703-872-9306	**Pages:**	14
Phone:		**Date:**	April 20, 2004
Re:	09/942,515	**CC:**	

Applicants: Bhullar et al.
Serial No.: 09/942,515
Filed: August 29, 2001
Entitled: BIOSENSOR WITH CODE PATTERN (as amended)
Ref. No.: RDID 00108 US
Examiner: Olsen, Kaj K
Group: 1753

Attachments:

- Transmittal Letter (1pp)
- Fee Transmittal (1pp)(duplicate)
- Amendment and Reply Under 37 CFR 1.111 (8pp)
- Petition for Extension of Time (1pp)(duplicate)
- Fax Transmittal (1pp)

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PAGE 1/14 * RCVD AT 4/20/2004 3:03:23 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/11 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):04-34

PTO/SB/21 (02-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/942,515
Filing Date	8-29-2001
First Named Inventor	Bhullar
Art Unit	1753
Examiner Name	Olsen
Attorney Docket Number	RDID 00108 US

Total Number of Pages in This Submission: 14

ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
  - [ ] Fee Attached
- [x] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] After Allowance communication to Technology Center (TC)
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please identify below): Fax Transmittal

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	The Law Office of Jill L. Woodburn, L.L.C. Jill L. Woodburn
Signature	Jill L. Woodburn
Date	April 20, 2004

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Typed or printed name	Jill L. Woodburn		
Signature	Jill L. Woodburn	Date	April 20, 2004

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

AMENDMENTS TO THE SPECIFICATION

At page 1 line 1 please change the title of the application as follows:

Biosensor with code pattern

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AMENDMENTS TO THE CLAIMS

1. (Currently amended) A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a bar code pattern, wherein there is sufficient contrast between the conductive coating and the substrate such that the bar code pattern is optically discernible from the substrate in order to identify the bar code pattern, and

at least one reagent positioned on at least one electrode.

2. (Cancelled).

3. (Original) The biosensor of claim 1 wherein the conductive coating is gold.

4. (Cancelled).

5. (Currently amended) The biosensor of claim 1 further comprising a second electrically ~~conducive~~ conductive coating positioned on the code pattern.

6. (Currently amended) The biosensor of claim 1 wherein the code pattern is electrically discernible from the substrate in order to identify the code pattern.

7. (Original) The biosensor of claim 1 wherein the code pattern includes recesses formed in the conductive coating.

8. (Original) The biosensor of claim 1 wherein the support substrate includes opposite first and second ends and the code pattern is positioned adjacent to the second end.

9. (Original) The biosensor of claim 1 further comprising a cover substrate extending across at least a portion of the electrodes.

10. (Original) The biosensor of claim 1 wherein code pattern includes pads that are spaced-apart from the surrounding electrically conductive coating.

11. (Original) The biosensor of claim 10 wherein the pads are isolated from one another.

12. (Original) The biosensor of claim 10 wherein the pads are interconnected.

13. (Currently amended) A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and a bar code pattern, wherein there is sufficient

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contrast between the conductive coating and the substrate such that the code pattern is optically discernible from the substrate in order to identify the bar code pattern, and

a cover cooperating with the support substrate to define a channel and at least a portion of the electrodes are positioned in the channel.

14. (Cancelled).

15. (Currently amended) The biosensor of claim 13 further comprising a second electrically ~~conducive~~ conductive coating positioned on the code pattern.

16. (Currently amended) The biosensor of claim 13 wherein the code pattern is electrically discernible from the substrate in order to identify the bar code pattern.

17. (Original) The biosensor of claim 13 wherein the support substrate includes opposite first and second ends and the code pattern is positioned adjacent to the second end.

18. (Original) The biosensor of claim 17 wherein the electrodes cooperate to define an array positioned adjacent to the first end.

19. (Original) The biosensor of claim 18 wherein the channel extends from the first end to the array.

20. (Cancelled).

21. (Currently amended) The biosensor of claim ~~20~~ 13 wherein the conductive coating is gold.

22. (Original) The biosensor of claim 13 wherein code pattern includes pads that are spaced-apart from the surrounding electrically conductive coating.

23. (Original) The biosensor of claim 22 wherein the pads are isolated from one another.

24. (Original) The biosensor of claim 22 wherein the pads are interconnected.

Claims 25-29 (Cancelled).

30. (Currently amended) A biosensor comprising:

a support substrate,

an electrically conductive coating positioned on the support substrate, the coating being formed to define electrodes and means for identifying the biosensor, wherein there is sufficient contrast between the conductive coating and the substrate such that the ~~identifying~~ means for identifying the biosensor is optically discernible from the substrate

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in order to identify the means for identifying the biosensor, wherein the means for identifying the biosensor is a bar code.

31. (Original) The biosensor of claim 30 further comprising a cover that cooperates with the support substrate to define a channel and at least a portion of the electrodes are positioned in the channel.

32. (Cancelled).

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REMARKS

Claims 1, 3, 5-13, 15-19, 21-24, and 30-31 are pending in the present application. Claims 1, 13, and 30 are independent claims.

The title of the application is objected to as not aptly descriptive. The title is amended to add the phrase "with code pattern". As amended, the title is believed to be adequately descriptive. Withdrawal of the objection to the title is respectfully requested.

Claims 1-24, and 30-32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite. Claims 2, 4, 14, 20, and 32 have been cancelled without prejudice or disclaimer of the subject matter recited therein. The rejection is traversed in light of the amendments to claims 1, 13 and 30. Support for the amendments is found in the specification, particularly at page 2 lines 30-31, page 5 line 3, page 9 lines 16 and 21-25, and Figures 1-2. No new matter is added by virtue of the amendments. The rejection sets forth several points, which will be addressed below.

Regarding claims 1, 13, and 30:

The rejection proffers that it is unclear what the metes and bounds of a "discernible" code pattern would be. Claims 1 and 13 are amended to recite that the bar code pattern is optically discernible from the substrate in order to identify the bar code pattern. Claim 30 is amended to recite that the means for identifying the biosensor is optically discernible from the substrate in order to identify the means for identifying the biosensor. The claims as amended are believed to be sufficiently definite.

Regarding claim 13:

The rejection proffers that it is it is unclear what a "sufficient contrast" would be. As discussed above, claim 13 has been amended to recite that that the bar code pattern is optically discernible from the substrate in order to identify the bar code pattern. As such, the claim as amended is believed to be sufficiently definite.

Regarding claim 30:

The rejection proffers that the presence of "mean for identifying" and later reference to an "identifying means" is potentially confusing. As such, claim 30 has been amended to recite consistent use of the term "means". The claim as amended is believed to be sufficiently definite.

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Regarding dependent claims 5 and 15:

The claims are amended to correct a typographical error.

Regarding dependent claims 6 and 16:

Claims 6 and 16 are amended to recite that the bar code pattern is electrically discernible from the substrate in order to identify the bar code pattern.

In light of the above amendments, the claims are believed to be sufficiently definite for purposes of 35 U.S.C. 112, second paragraph. Reconsideration of the rejection in light of the amendments, leading to withdrawal of the rejection and allowance of the claims is respectfully requested.

The Examiner's statement that claims 2, 20, and 32 would be allowable if rewritten to overcome the rejection(s) under 35 U.S.C. 112, second paragraph, set forth in the Office action and to include all of the limitations of the base claim and any intervening claims is acknowledged. Independent claims 1, 13, and 30 have been amended to overcome the rejection(s) under 35 U.S.C. 112, second paragraph and to include the limitations of claims 2, 20, and 32 respectively. As such, allowance of independent claims 1, 13, and 30 is respectfully requested.

Claims 1, 4-19, 22-24, 30 and 31 are rejected under 35 U.S.C. 102(e) as being anticipated by Kawanaka et al. (USP 6,599,406 B1) [hereinafter Kawanaka]. Claims 4 and 14 are cancelled without prejudice or disclaimer of the subject matter recited therein.

As discussed above, claims 1, 13, and 30 have been amended to include the limitations of allowable claims 2, 20, and 32 respectively. Claims 5-12 depend from amended claim 1, claims 15-19 and 22-24 depend from amended claim 13, and claim 31 depends from amended claim 30. As such, the claims as amended are not anticipated and believed to be patentable over Kawanaka et al. Reconsideration of the rejections, leading to withdrawal of the rejections and allowance of the claims is respectfully requested.

Claims 3 and 21 (and claims 5 and 15 in the alternative) are rejected under 35 U.S.C. 103(a) as being obvious Kawanaka et al. (USP 6,599,406 B1) [hereinafter Kawanaka] in view of Song et al. (USP 5,567,302).

As discussed above, claims 1 and 13 have been amended to include the limitations of allowable claims 2 and 20 respectively. Claim 3 depends from amended claim 1 and claim 21 depends from amended claim 13. Accordingly, the claimed invention meets the

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test of patentability under 35 U.S.C. 103(a). Entry of the amendments leading to reconsideration of the rejection of the claims and withdrawal of the rejection is respectfully requested.

Reconsideration of the rejections, leading to withdrawal of the rejections and allowance of the claims is respectfully requested.

This application is deemed to be in condition for allowance and as such is respectfully requested. In addition, it is requested that this paper be considered as a Petition for an Extension of Time sufficient to effect a timely response and fees be charged to Deposit Account No. 02-2958 with reference to (RDID 00108 US).

Respectfully submitted,

Date: April 20, 2004

Jill L. Woodburn, Reg. No. 39,874
The Law Office of Jill L. Woodburn, L.L.C.
128 Shore Drive
Ogden Dunes, IN
Telephone No.: 219-764-4005
Facsimile No.: 219-764-4070

PAGE 12/14 * RCVD AT 4/20/2004 3:03:23 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/11 * DNIS:8729306 * CSID:2197644070 * DURATION (mm-ss):04-34

PTO/SB/22 (08-03)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional) RDID 00108 US

In re Application of Bhullar et al.
Application Number 09/942,515 Filed 8-29-2001
For Biosensor with code pattern (as amended)
Art Unit 1753 Examiner Olsen

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

- [X] One month (37 CFR 1.17(a)(1)) $ 110
- [ ] Two months (37 CFR 1.17(a)(2)) $
- [ ] Three months (37 CFR 1.17(a)(3)) $
- [ ] Four months (37 CFR 1.17(a)(4)) $
- [ ] Five months (37 CFR 1.17(a)(5)) $

[ ] Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee amount shown above is reduced by one-half, and the resulting fee is: $ _____.

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[X] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account Number 02-2958.

I have enclosed a duplicate copy of this sheet.

I am the
- [ ] applicant/inventor.
- [ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).
- [X] attorney or agent of record. Registration Number 39874
- [ ] attorney or agent under 37 CFR 1.34(a). Registration number if acting under 37 CFR 1.34(a) _____.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

April 20, 2004 — Date
Joe L. Woodburn — Signature

219-764-4005 — Telephone Number
Jill L. Woodburn — Typed or printed name

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

[X] Total of 1 (duplicate) forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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PTO/SB/17 (10-03)
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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 110.00

Complete if Known	
Application Number	09/942,515
Filing Date	8-29-2001
First Named Inventor	Bhullar
Examiner Name	Olsen
Art Unit	1753
Attorney Docket No.	RDID 00108 US

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 02-2958

Deposit Account Name: Roche Diagnostics Corporation

The Director is authorized to: *(check all that apply)*

☒ Charge fee(s) indicated below ☒ Credit any overpayments

☐ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 0

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims	22 -20** =	0	X	= 0
Independent Claims	3 - 3** =		X	= 0
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	110
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 110.00

SUBMITTED BY				(Complete if applicable)	
Name (Print/Type)	Jill L. Woodburn	Registration No. (Attorney/Agent)	39874	Telephone	219764-4005
Signature	Jill L. Woodburn			Date	April 20, 2004

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 2003

Application or Control Number: 09/942515

CLAIMS AS FILED - PART I

TOTAL CLAIMS		
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL INDEPENDENT CLAIMS	minus 20=	
INDEPENDENT CLAIMS	minus 3 =	
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	355.00	OR	BASIC FEE	710.00
XS 9=		OR	XS18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL		OR	TOTAL	

4/20/04

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 22	Minus	** 32	= 0
Independent	* 3	Minus	*** 3	= 0
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
XS 9=		OR	XS18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
XS 9=		OR	XS18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
XS 9=		OR	XS18=	
X40=		OR	X80=	
+135=		OR	+270=	
TOTAL ADD'T FEE		OR	TOTAL ADD'T FEE	

PTO/SB/22 (08-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARMENT OF COMMERCE
Under the paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) | Docket Number (Optional) RDID 00108 US

In re Application of Bhullar et al.
Application Number 09/942,515 | Filed 8-29-2001
For Biosensor with code pattern (as amended)
Art Unit 1753 | Examiner Olsen

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

- [X] One month (37 CFR 1.17(a)(1)) $ 110
- [ ] Two months (37 CFR 1.17(a)(2)) $
- [ ] Three months (37 CFR 1.17(a)(3)) $
- [ ] Four months (37 CFR 1.17(a)(4)) $
- [ ] Five months (37 CFR 1.17(a)(5)) $

- [ ] Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee amount shown above is reduced by one-half, and the resulting fee is: $ _____.
- [ ] A check in the amount of the fee is enclosed.
- [ ] Payment by credit card. Form PTO-2038 is attached.
- [X] The Director has already been authorized to charge fees in this application to a Deposit Account.
- [X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account Number 02-2958.

I have enclosed a duplicate copy of this sheet.

I am the
- [ ] applicant/inventor.
- [ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).
- [X] attorney or agent of record. Registration Number 39874
- [ ] attorney or agent under 37 CFR 1.34(a). Registration number if acting under 37 CFR 1.34(a) _____.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

April 20, 2004 — Date
Joe L. Woodburn — Signature
219-764-4005 — Telephone Number
Jill L. Woodburn — Typed or printed name

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

[X] Total of 1 (duplicate) forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

fee processed

04/30/2004 VSEAFORT 00000001 09942515
01 FC:1251 110.00 DA

BEST AVAILABLE COPY

PTO/SB/06 (08-03)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 09/942,515

CLAIMS AS FILED – PART I

	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE (37 CFR 1.16(a))				$	OR		$
TOTAL CLAIMS (37 CFR 1.16(c))	32 minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(b))	4 minus 3 =	*	X $ =		OR	X $ =	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(d))			+ $ =		OR	+ $ =	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL		OR	TOTAL	

CLAIMS AS AMENDED – PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total (37 CFR 1.16(c))	* 22	Minus	** 32	= –	X $ =		OR	X $ =	
Independent (37 CFR 1.16(b))	*	Minus	*** 4	= –	X $ =		OR	X $ =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $ =		OR	+ $ =	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total (37 CFR 1.16(c))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(b))	*	Minus	***	=	X $ =		OR	X $ =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $ =		OR	+ $ =	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total (37 CFR 1.16(c))	*	Minus	**	=	X $ =		OR	X $ =	
Independent (37 CFR 1.16(b))	*	Minus	***	=	X $ =		OR	X $ =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $ =		OR	+ $ =	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

32842 7590 07/06/2004

THE LAW OFFICE OF JILL L. WOODBURN, L.L.C.
JILL L. WOODBURN
128 SHORE DR.
OGDEN DUNES, IN 46368

EXAMINER	
OLSEN, KAJ K	
ART UNIT	PAPER NUMBER
1753	

DATE MAILED: 07/06/2004

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US	8155

TITLE OF INVENTION: BIOSENSOR

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	10/06/2004

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE REFLECTS A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE APPLIED IN THIS APPLICATION. THE PTOL-85B (OR AN EQUIVALENT) MUST BE RETURNED WITHIN THIS PERIOD EVEN IF NO FEE IS DUE OR THE APPLICATION WILL BE REGARDED AS ABANDONED.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 07/04) Approved for use through 04/30/2007.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (703) 746-4000

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

32842 7590 07/06/2004

THE LAW OFFICE OF JILL L. WOODBURN, L.L.C.
JILL L. WOODBURN
128 SHORE DR.
OGDEN DUNES, IN 46368

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US	8155

TITLE OF INVENTION: BIOSENSOR

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	10/06/2004

EXAMINER	ART UNIT	CLASS-SUBCLASS
OLSEN, KAJ K	1753	204-403010

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent); ❑ individual ❑ corporation or other private group entity ❑ government

4a. The following fee(s) are enclosed:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s):

❑ A check in the amount of the fee(s) is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

❑ b. Applicant is not claiming SMALL ENTITY status. See, e.g., 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

(Authorized Signature) (Date)

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE(S)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US	8155

32842 7590 07/06/2004

THE LAW OFFICE OF JILL L. WOODBURN, L.L.C.
JILL L. WOODBURN
128 SHORE DR.
OGDEN DUNES, IN 46368

EXAMINER	
OLSEN, KAJ K	

ART UNIT	PAPER NUMBER
1753	

DATE MAILED: 07/06/2004

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 357 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 357 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (703) 305-1383. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

Notice of Allowability	Application No.	Applicant(s)	
	09/942,515	BHULLAR ET AL.	
	Examiner	Art Unit	
	Kaj K Olsen	1753	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment of 4-20-2004*.

2. ☒ The allowed claim(s) is/are *1,3,5-13,15-19,21-24,30 and 31*.

3. ☒ The drawings filed on *8-29-2001* are accepted by the Examiner.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
   1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☒ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

EXAMINER'S AMENDMENT

1. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

The application has been amended as follows: On page 4, line 6, after "1999, ", the phrase --now US Patent 6,662,439, -- was inserted.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kaj Olsen whose telephone number is (571) 272-1344. The examiner can normally be reached on Monday through Thursday from 6:30 A.M. to 4:00 P.M. and on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Nam Nguyen, can be reached on 571-272-1342. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



Kaj Olsen Ph.D.
Primary Examiner
AU 1753
June 29, 2004

Issue Classification	Application No.	Applicant(s)	
	09/942,515	BHULLAR ET AL.	
	Examiner	Art Unit	
	Kaj K Olsen	1753	

ISSUE CLASSIFICATION

ORIGINAL CLASS	ORIGINAL SUBCLASS	CROSS REFERENCE(S) CLASS	CROSS REFERENCE(S) SUBCLASS (ONE SUBCLASS PER BLOCK)
204	403.01	204	403.02

INTERNATIONAL CLASSIFICATION

G	01	N	27	/327
				/
				/
				/
				/

(Assistant Examiner) (Date)

Wanda Mitchell
(Legal Instruments Examiner) (Date) 7.01.04

Kaj K. Olsen
Kaj K. Olsen 6/29/04
(Primary Examiner) (Date)

Total Claims Allowed: 22

O.G. Print Claim(s)	O.G. Print Fig.
1	1

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	22	31		61		91		121		151		181
	2		32		62		92		122		152		182
2	3		33		63		93		123		153		183
	4		34		64		94		124		154		184
3	5		35		65		95		125		155		185
4	6		36		66		96		126		156		186
5	7		37		67		97		127		157		187
6	8		38		68		98		128		158		188
7	9		39		69		99		129		159		189
8	10		40		70		100		130		160		190
9	11		41		71		101		131		161		191
10	12		42		72		102		132		162		192
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16	19		49		79		109		139		169		199
	20		50		80		110		140		170		200
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19	23		53		83		113		143		173		203
20	24		54		84		114		144		174		204
	25		55		85		115		145		175		205
	26		56		86		116		146		176		206
	27		57		87		117		147		177		207
	28		58		88		118		148		178		208
	29		59		89		119		149		179		209
21	30		60		90		120		150		180		210

Search Notes

Application No.	Applicant(s)	
09/942,515	BHULLAR ET AL.	
Examiner	Art Unit	
Kaj Olsen	1753	

SEARCHED

Class	Subclass	Date	Examiner
204	403.01	12/12/03	KKO
↓	403.02	↓	↓
422	82.01	↓	↓
updated		6-29-04	KKO

INTERFERENCE SEARCHED

Class	Subclass	Date	Examiner
204	403.01	6-29-04	KKO
↓	403.02	↓	↓

SEARCH NOTES (INCLUDING SEARCH STRATEGY)

	DATE	EXMR
inventor name search	12/12/03	KKO
BRS search of USPAT database	12/12/03	KKO
STN search of USPAT, ANABSTR	6-29-04	KKO

OIPE JC73 OCT 06 2004 PATENT & TRADEMARK OFFICE

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail

Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

or Fax (703) 746-4000

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

32842 7590 07/06/2004

THE LAW OFFICE OF JILL L. WOODBURN, L.L.C.
JILL L. WOODBURN
128 SHORE DR.
OGDEN DUNES, IN 46368

10/06/2004 JADD02 00000040 022958 09942515

01 FC:1501 1370.00 DA
02 FC:1504 300.00 DA
03 FC:8001 [illegible] DA

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

JILL L. WOODBURN	(Depositor's name)
Jill L. Woodburn	(Signature)
10-6-2004	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US	8155

TITLE OF INVENTION: BIOSENSOR

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1330	$300	$1630	10/06/2004

EXAMINER	ART UNIT	CLASS-SUBCLASS
OLSEN, KAJ K.	1753	204-403010

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 JILL L. WOODBURN
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: ROCHE DIAGNOSTICS CORPORATION

(B) RESIDENCE: (CITY and STATE OR COUNTRY): INDIANAPOLIS, IN

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ individual ☒ corporation or other private group entity ☐ government

4a. The following fee(s) are enclosed:

☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☒ Advance Order - # of Copies 10

4b. Payment of Fee(s):

☐ A check in the amount of the fee(s) is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number 02-0958 (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

☐ b. Applicant is not claiming SMALL ENTITY status. See, e.g., 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

(Authorized Signature) Jill L. Woodburn (Date) 10-6-2004

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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TRANSMIT THIS FORM WITH FEE(S)

PAGE 2/3 * RCVD AT 10/6/2004 8:43:45 AM [Eastern Daylight Time] * SVR:USPTO-EFXRF-2/1 * DNIS:7464000 * CSID:2197644070 * DURATION (mm-ss):01-34

OIPE JC73
OCT 0 6 2004
PATENT & TRADEMARK OFFICE

Fax

To:	Mail Stop Issue Fee Commissioner for Patents	**From:**	Jill Woodburn
Fax:	703-746-4000	**Pages:**	3
Phone:		**Date:**	October 6, 2004
Re:	09/942,515	**CC:**	

Applicants: Bhullar et al.
Serial No.: 09/942,515
Filed: August 29, 2001
Entitled: BIOSENSOR WITH CODE PATTERN
Ref. No.: RDID 00108 US
Examiner: Olsen, Kaj K
Group: 1753

Attachments:

- Transmittal Form (1pp)(in duplicate)
- Fax Cover Sheet (1pp)

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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:

☒ Practitioners associated with the Customer Number: **23690**

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

☒ The address associated with Customer Number: **23690**

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

Assignee Name and Address:

Roche Diagnostics Operations, Inc.
9115 Hague Road
Indianapolis, Indiana 46250

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record
The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	D. Michael Young	Date March 22, 2006
Name	D. Michael Young	Telephone 317-521-7340
Title	Assistant Secretary	

PTO/SB/96 (11-05)
Approved for use through 7/31/2006. OMB 0651-0031
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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Roche Diagnostics Operations, Inc.
Application No./Patent No: 6,814,844 Filed/Issue Date: November 9, 2004
Entitled: Biosensor
Roche Diagnostics Operations, Inc. (Name of Assignee) a, Corporation (Type of Assignee, e.g., Corporation, Partnership, University, Government Agency, etc.)

States that it is:

1. ☒ The assignee of the entire right, title, and interest; or

2. ☐ an assignee of less than the entire right, title, and interest.
The extent (by percentage) of its ownership interest is: _____%.

In the patent application/patent identified above by virtue of either:

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____ , or for which a copy thereof is attached.

OR

B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: Bhullar, Austera, Walling, Ranney, Pauley, Jr. To: Roche Diagnostics Corporation
The document was recorded in the United States Patent and Trademark Office at Reel 013950, Frame 0607, or for which a copy thereof is attached.

2. From: Groll To: Roche Diagnostics GmbH
The document was recorded in the United States Patent and Trademark Office at Reel 013950, Frame 0490, or for which a copy thereof is attached.

3. From: Roche Diagnostics GmbH To: Roche Diagnostics Corporation
The document was recorded in the United States Patent and Trademark Office at Reel 014002, Frame 0966, or for which a copy thereof is attached.

4. From: Roche Diagnostics Corporation To: Roche Diagnostics Operations, Inc.
The document was recorded in the United States Patent and Trademark Office at Reel 015215, Frame 0061, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

☐ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or currently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Justin L. Sage/	July 10, 2008
Signature	Date
Justin L. Sage	317-521-7503
Typed or printed name	Telephone Number
Appointed Practitioner to Act on Behalf of Assignee	
Customer Number 23690	
Title	

Electronic Acknowledgement Receipt

EFS ID:	3597249
Application Number:	09942515
International Application Number:	
Confirmation Number:	8155
Title of Invention:	BIOSENSOR
First Named Inventor/Applicant Name:	Raghbir S. Bhullar
Customer Number:	32842
Filer:	Justin L. Sage
Filer Authorized By:	
Attorney Docket Number:	RDID 00108US
Receipt Date:	10-JUL-2008
Filing Date:	29-AUG-2001
Time Stamp:	16:21:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes) /Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	POA.pdf	649134 a5044f4451bf16c7af936271a893b23e08e40b5f	no	1

Warnings:	
Information:	

2	Assignee showing of ownership per 37 CFR 3.73(b).	Assignee_Statement.pdf	663028 f348b35a3b7de97f9415a56977e1ac3cbc9d92bb	no	1

Warnings:	
Information:	
Total Files Size (in bytes):	1312162

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US

CONFIRMATION NO. 8155

23690
ROCHE DIAGNOSTICS OPERATIONS INC.
9115 Hague Road
Indianapolis, IN 46250-0457

POA ACCEPTANCE LETTER

OC00000031010470

Date Mailed: 07/17/2008

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 07/10/2008.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/mteklemichael/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
09/942,515	08/29/2001	Raghbir S. Bhullar	RDID 00108US

CONFIRMATION NO. 8155

POWER OF ATTORNEY NOTICE

32842
The Law Office of Jill L. Woodburn, LLC
128 Shore Dr.
Portage, IN 46368

OC000000031010432

Date Mailed: 07/17/2008

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 07/10/2008.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

/mteklemichael/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101