

Commercial Biosensors

Applications to Clinical, Bioprocess,
and Environmental Samples

Edited by

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A WILEY-INTERSCIENCE PUBLICATION

JOHN WILEY & SONS, INC.

New York / Chichester / Weinheim / Brisbane / Singapore / Toronto

ROCHE 1031

Cover photo courtesy of i-STAT Corporation

Chemistry Library
University of Wisconsin - Madison
2381 Chemistry Building
1101 University Avenue
Madison, WI 53706-1398

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Published simultaneously in Canada.

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Library of Congress Cataloging-in-Publication Data:

Commercial biosensors : applications to clinical, bioprocess, and environmental samples / edited by Graham Ramsay.

p. cm.—(Chemical analysis : v. 148)

Includes index.

ISBN 0-471-58505-X (cloth : alk. paper)

I. Biosensors. I. Ramsay, Graham, 1954— II. Series.

R857.B54C65 1998

616.07'5—dc21

97-28054

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

PREFACE

Over the past 15 years there has been intense research on biosensors, but few ideas other than the glucose biosensor have made the difficult transition from research laboratory to commercially available product. However, in the past few years the number of commercialized biosensors has greatly increased, notably due to the introduction of i-STAT's hand-held, point-of-care system and the advent of surface plasmon resonance devices (e.g., Pharmacia's BIAcore) and evanescent wave devices (e.g., Fisons Applied Sensor Technology's IAsys). This book presents applications of some of these to clinical, bioprocess, and environmental samples and is intended to be of interest to students, teachers, and research workers in the biosensor field.

It is arranged in three sections, dealing with applications to clinical, bioprocess, and environmental samples, respectively. The first section describes the use of biosensors for in-home diabetes monitoring, point-of-care diagnostics, noninvasive sensing, and the use of surface plasmon resonance/evanescent wave instruments. The second section illustrates the use of biosensors for bioprocess control, for example, by measuring glucose, sucrose, glutamate, or choline concentrations during the production of foods and beverages and by measuring the concentration of ethanol during beer fermentations. The final section covers the use of biological oxygen demand (BOD) biosensors for monitoring environmental samples such as wastewater.

Many definitions exist for a biosensor, but the one used here is as follows: A biosensor is a chemical or mass sensor in which the analytical signal is generated by a biocomponent immobilized on, or in close proximity to, a transducer.

As this book goes to press, a new generation of biosensors for DNA analysis has reached the marketplace. These DNA chips (or genosensors) come in two configurations: In Format I a dense microarray of single-stranded, complementary DNA targets is immobilized on a glass substrate approximately 1 cm² and interrogated by labeled, single-stranded DNA probes derived from total cellular mRNA. Conversely, in Format II, sample DNA labeled with a fluorophore is added to a high-density microarray of oligonucleotide probes. Following stringent hybridization and washing, the analytical signal is measured for each array element and processed by computer to yield hybridization data. Applications have included massively parallel gene discovery and gene expression monitor-

ing, detection of BRCA1 cancer, HIV-1, β -thalassemia and cystic fibrosis mutations, and gene mapping.

DNA chip technology is commercially available from Affymetrix, Inc., Santa Clara, California (GeneChip™ for detection of HIV or p53 gene mutations), Hyseq, Inc., Sunnyvale, California (HyChip™-based assays) and Synteni, Inc., Fremont, California (Gene Expression Microarray [GEM™] assays). Nanogen, Inc., San Diego, California (Automated Programmable Electronic Matrix, [APEX™]), and Molecular Dynamics, Sunnyvale, California are developing proprietary systems for commercialization.

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PART

I

APPLICATIONS TO CLINICAL SAMPLES

CHAPTER

1

BIOSENSORS FOR PERSONAL DIABETES MANAGEMENT

TIMOTHY P. HENNING and DAVID D. CUNNINGHAM

1. Introduction

- 1.1. Diabetes
- 1.2. Diabetes Control and Complications Trial
- 1.3. Testing by Diabetics
- 1.4. Objectives

2. Theory of Operation

- 2.1. Reflectance-Based Methods
- 2.2. Electrochemical Methods
- 2.3. Enzymes and Reagents
- 2.4. Hexokinase Method
- 2.5. Glucose Oxidase–Peroxidase Optical Method
- 2.6. Glucose Oxidase–Organic Mediator Optical Method
- 2.7. Glucose Oxidase–Prussian Blue Method
- 2.8. Glucose Oxidase–Ferricyanide Electrochemical Method
- 2.9. Glucose Oxidase–Ferrocene Electrochemical Method
- 2.10. Reusable Sensors

3. Meters

- 3.1. Meter Introduction
- 3.2. Bayer Glucometer Encore
- 3.3. LifeScan One Touch
- 3.4. LifeScan Basic and Profile Operation
- 3.5. One Touch Basic
- 3.6. One Touch Profile
- 3.7. SureStep
- 3.8. Boehringer Mannheim Accu-Chek Instant
- 3.9. Boehringer Mannheim Accu-Chek Easy
- 3.10. Bayer Elite
- 3.11. Boehringer Mannheim Accu-Chek Advantage
- 3.12. MediSense
- 3.13. ExacTech

Commercial Biosensors: Applications to Clinical, Bioprocess, and Environmental Samples, Edited by
Graham Ramsay.

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- 3.14. ExacTech RSG
- 3.15. Companion 2
- 3.16. Precision QID
- 4. **Performance**
 - 4.1. Performance Introduction
 - 4.2. Accuracy
 - 4.3. Precision
 - 4.4. Chemistry
 - 4.5. User Interactions
 - 4.6. Physiology
 - 4.7. Environmental Issues
 - 4.8. Performance Summary
- 5. **Future Directions**

1. INTRODUCTION

1.1. Diabetes

There are an estimated 8 million diabetics in the United States today and an estimated 8 million diabetics who have not been diagnosed. The amount of money spent on treating diabetics is close to that spent on heart disease and cancer. Because of the crisis in health care costs in the United States, diabetes is now receiving the attention of the U.S. government and health care providers. An important area that is expected to bring down the cost of diabetes is home testing by diabetics using a glucose biosensor. The glucose biosensor is undoubtedly the most financially successful sensor of any type. The glucose biosensor can be looked at as a model for the development of future biosensors for home health care.

Diabetes is a disease in which the body is no longer able to regulate the level of glucose in the blood. Using the simplest model of how the body regulates glucose, the adsorption of glucose from food results in an elevation of glucose in the blood. The islet cells in the pancreas are stimulated by the glucose level to secrete the hormone insulin. Insulin acts on the cells of the body to take up the glucose. If a shortage is felt by the body, insulin production is slowed and the liver releases glucose that is stored as glycogen. In a person without diabetes the body is able to regulate the amount of glucose in the blood very tightly. This is very important because the primary source of energy for the brain as well as all other cells in the body is glucose. In diabetes the autoregulation mechanisms of glucose fail. The consequences of poor glucose regulation are at best, long-term damage to organs from too much glucose (hyperglycemia), and at worst, coma or death caused by too little glucose reaching the brain (hypoglycemia).

There are two forms of diabetes. *Type 1 diabetes*, sometimes referred to as *juvenile diabetes*, usually strikes children and young adults. The insulin-producing islet cells in the pancreas are destroyed by the diabetic's own immune system. The type 1 diabetic usually loses all insulin-producing capability and must inject themselves with insulin before each meal to allow the body to utilize the glucose that is adsorbed from the food. The type 1 diabetic may also be referred to as having *insulin-dependent diabetes mellitus* (IDDM). The *type 2 diabetic* usually becomes diabetic at a much older age than the type 1 diabetic. Type 2 diabetes is very complex because it is not just caused by loss of insulin production. Type 2 diabetics can usually increase their glucose regulation by losing weight. Initially, most type 2 diabetics are treated by diet control and with drugs that help the body metabolize glucose. Type 2 diabetics can be referred to as having *non-insulin-dependent diabetes mellitus* (NIDDM). The type 2 diabetic over time may need to start using insulin injections to maintain glucose regulation.

Diabetic's long- and short-term health relies on their ability to regulate their blood glucose levels. The method of choice for monitoring a diabetic's immediate condition is for patients to determine their own blood glucose level using a glucose biosensor. Such a biosensor must be accurate, cheap, portable, and simple enough to be used by the average person.

1.2. Diabetes Control and Complications Trial

A study was begun in 1983 to examine the effect of tight regulation of glucose levels on the complications experienced by type 1 diabetics [1]. The Diabetes Control and Complications Trial (DCCT) involved over 1441 diabetics and spanned a period of 10 years. The study followed a group of diabetics who used a standard regime of glucose measurements and insulin shots and a group who monitored their glucose more often and did more frequent insulin injections, leading to tighter glucose control. Over time, the group of diabetics who maintained tight glucose control had significantly fewer diabetes-related complications than did the group using the standard regime. A computer simulation was done based on the DCCT results that estimated an additional 5 years of life, 8 years of sight, 6 years free from kidney disease, and 6 years free of amputations for a diabetic following tight glucose control versus the standard regiment [2]. The DCCT study clearly showed that frequent glucose monitoring by the diabetic resulted in a healthier life.

Several factors have combined over the past few years to make glucose biosensors one of the most financially attractive areas in medical diagnostics. The DCCT showed that frequent monitoring could reduce the complications of diabetes and correspondingly, the cost of diabetes. The increase in health care costs worldwide has placed new emphasis on each diagnostic test being cost-justified. The population in many developed countries is aging, which leads to a

dramatic increase in type 2 diabetes. All these factors have created a rapidly expanding market for glucose monitoring which will probably last well beyond the year 2000.

1.3. Testing by Diabetics

Next we describe how a diabetic would normally run a glucose biosensor. The test involves much more than a chemical reaction on a strip. The interactions of the user with the test are vitally important to achieving an accurate result. Since this test may be performed by people who know nothing about medical diagnostics, the steps required to perform an accurate test become even more important. The testing begins by a diabetic assembling all the parts necessary for a test. He or she usually carries a wallet-sized case which contains a hand-held meter, lancets and a lancet device, test strips (biosensors), cotton wipes, and alcohol wipes. The diabetic washes his or her hands to remove surface glucose as well as to wash away germs. Warm water is recommended to increase the blood flow in the hand. The finger to be used for the blood draw may be wiped with an alcohol swab to decrease the chance of infection. A lancet is loaded into a lancet device and placed against the top and the side of the finger. The lancet device is actuated, which causes the lancet to pierce the skin. The amount of blood obtained by lancing is usually not sufficient to run the glucose biosensor. The diabetic will apply pressure to the hand or finger, squeezing out a sufficient quantity of blood to run the biosensor. The blood on the finger is then touched to the glucose biosensor. The biosensor then begins testing the blood. The diabetic then wipes the finger off with a cotton swab to remove the excess blood and possibly applies pressure to try to stop the bleeding. When the biosensor has completed the glucose assay, the diabetic writes the result in a logbook for future reference. The biosensor, cotton swab, and used lancet are disposed of and the remaining materials are packed up.

The human interactions involved in the running of a glucose biosensor make the development of a successful product even more difficult. The biosensor must be able to perform under a variety of real-world conditions. A successful glucose biosensor must also meet the diabetic's expectations. A successful product will require the diabetic to do as few steps as possible, and these must be easy to do. The diabetic also needs a small device to make it portable. To be commercially successful the choice of a biosensor will not be decided solely by which offers the best precision and accuracy. The area of biosensor performance in the hands of diabetics is explored in more detail in Section 4.

1.4. Objectives

The objectives of this chapter are to explain the theory of how home glucose meters work, the meters that are on the market, and the performance of these

meters. Based on the definition of a biosensor as a "chemical sensor in which the analytic signal is generated by the reaction of the analyte species with an immobilized biocomponent on or in close proximity to a transducer," both reflectance and electrochemical glucose test strips are considered to be biosensors. We use the standard terminology of referring to the glucose biosensor as a test strip in combination with a glucose meter. Home glucose testing has a long and interesting history but is not covered in this chapter. The home glucose testing market is changing rapidly, with new meters being introduced each year. Our emphasis is on the meters and test strips currently on the market. As new meters are introduced, the meter section of this chapter will become dated, but the theory and performance sections should remain relevant for a long time.

2. THEORY OF OPERATION

2.1. Reflectance-Based Methods

Color-forming chemistries are most conveniently measured by reflectance spectrometry. These optically based strips are constructed with various layers, which provide a support function, a reflective function, an analytical function, and a sample-spreading function, as illustrated in Figure 1. The support element, which serves as the foundation for the dry reagent, generally consists of a thin, rigid plastic material and may also contain the reflective function. However, the reflective function can also be introduced by adding reflective (or scattering) materials such as TiO_2 , BaSO_4 , MgO , and ZnO to the dry chemistry mixture. The analytical function of the strips is described in greater detail in following sections. The spreading function may be provided by fabrics, membranes, or paper, in conjunction with surfactants in the dry chemistry element. The purposes of the spreading function are to disperse a sample rapidly after application and to form a uniform sample concentration quickly on the analytically active portion of the strip. Semipermeable membranes, swellable films, and membrane filters have been used to separate clear plasma from the whole blood cells, thereby reducing the effect of sample color on the reflectance reading. Particularly useful for separation of plasma from whole blood are glass fibers with a diameter of 0.5 to 1.5 μm and a density of 0.1 to 0.5 g/cm^3 , loosely stapled, or in the form of papers, fleece, or felts [3,4]. Upon formation of the colored reaction product, the amount of diffuse light reflected from the analytical element decreases according to the equation

$$\% R = \frac{I_u}{I_s} R_s$$

where I_u is the reflected light from the sample, I_s the reflected light from a standard, and R_s the percent reflectivity of the standard. The relationship between

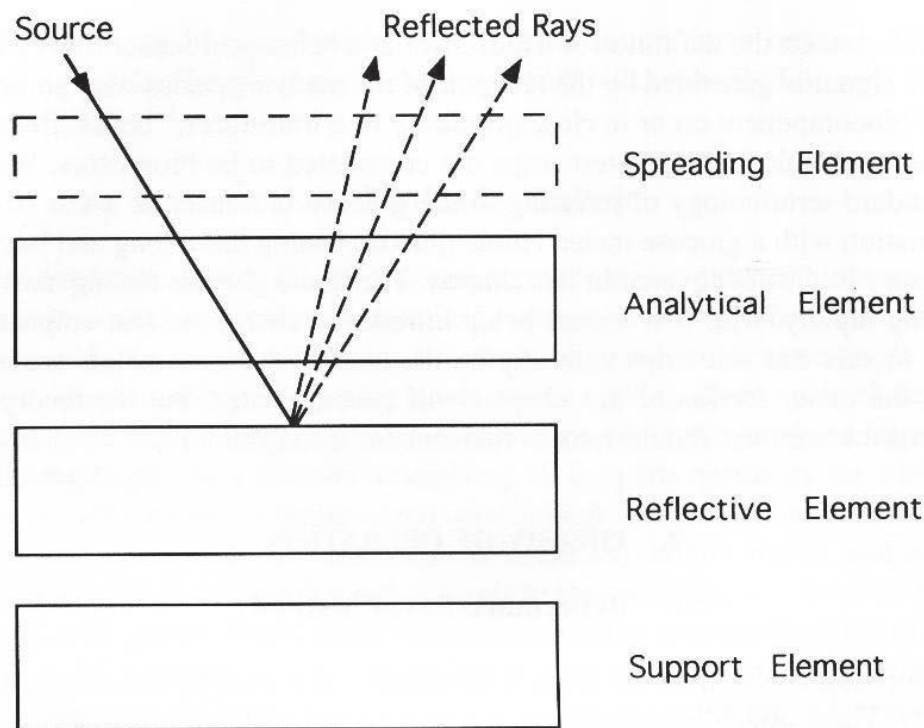


Figure 1. Basic functions of a reflectance-based test strip.

reflectance and concentration has been stated in more useful and convenient forms, such as the Kubelka–Munk and Williams–Clapper equations [5,6]. The Kubelka–Munk equation is

$$C \propto \frac{K}{S} = \frac{(1 - R)^2}{2R}$$

where C is concentration, K the absorption coefficient, S the scattering coefficient, and R the percent reflectance divided by 100.

2.2. Electrochemical Methods

Chemical reactions that involve electrochemically active molecules can be monitored using electrochemical techniques. An electrochemical measurement of a nonelectrochemically active analyte such as glucose starts with a chemical reaction involving the analyte. The chemical reaction needs to be selective for the analyte of interest, and this selectivity is normally supplied by an enzyme such as glucose oxidase. The product of the reaction of the analyte with the enzyme might be electroactive, or an additional electroactive component is added to regenerate the enzyme after its reaction with the analyte. The enzyme itself often undergoes an electron transfer reaction with the analyte, but enzymes are usually

not electroactive. The added electroactive component is called a mediator. In the case of an oxidase enzyme the mediator reoxidizes the enzyme, permitting one more analyte molecule to be oxidized. The mediator itself is then reoxidized at the electrode. The mediator is not consumed during the reaction and can cycle between the enzyme and the electrode many times. The reaction of the mediator with an electrode is determined by the energy of the electrons in the electrode. Electrons will flow between the mediator and electrode surface based on where they will have the lowest energy. The energy of the electrons in the mediator is fixed based on its chemical structure, but the energy of the electrons in the electrode is controlled by the applied potential and can be varied by varying the potential. The rate of the electron transfer reaction between the mediator and an electrode surface is given by the Butler–Volmer equation [7]. When the potential is large enough, all the mediator reaching the electrode reacts rapidly and the reaction becomes diffusion controlled. The current from a diffusion limited reaction follows the well-known Cottrell equation [8],

$$i = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}}$$

where i is the current, n the number of electrons, F is Faraday's constant, A is the electrode area, C the concentration, D the diffusion coefficient, and t the time. The current from a diffusion-controlled electrochemical reaction will decay away as the reciprocal square root of time. This means that the maximum electrochemical signal occurs at short times, as opposed to color-forming reactions, where the color becomes more intense with time. Several patents have been issued involving the use of the Cottrell equation for measuring glucose concentrations using biosensors [9,10].

The electrochemical method relies on measuring the current from the electron transfer between the electrode and the mediator. However, when a potential is first applied to the electrode, the dipole moments of solvent molecules will align with the electric field on the surface of the electrode, causing a current to flow. Thus at very short times this charging current interferes with the analytical measurement. Electrochemical sensors generally apply a potential to the electrode surface and measure the current after the charging current has decayed sufficiently.

2.3. Enzymes and Reagents

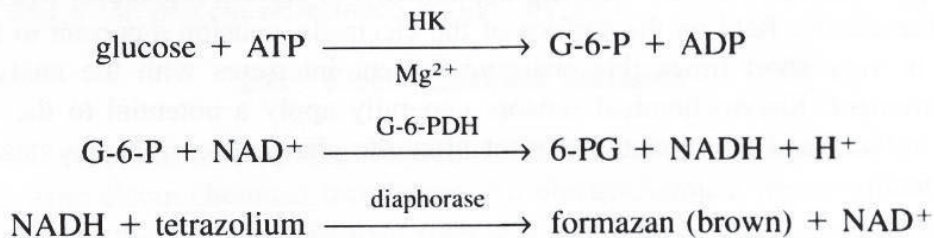
All of the commercially available test strips are based on enzymatic methods. Each enzyme is commercially available in various grades, some of which contain stabilizers, buffers, salt, and various levels of impurities [11–18]. One enzyme unit causes a reaction of 1 $\mu\text{mol}/\text{min}$. Each enzyme has an intrinsic stability (or

instability) to factors such as pH, temperature, physical sheer stress, organic solvents, and various other denaturing actions or agents. Naturally, some degree of inactivation is expected during the formulation and manufacturing steps as certain enzyme molecules are exposed to denaturing conditions and enzyme inhibitors. Additional inactivation is expected during the storage of the product. In general, sufficient enzyme is incorporated into the strip such that the assay reaction occurs in a conveniently short time. Similarly, the amounts of the other reagents formulated into the strips are in excess of the amount necessary for complete reaction with the glucose in the sample of blood.

Reagent formulations often include thickening agents, builders, emulsifiers, dispersion agents, pigments, plasticizers, pore formers, wetting agents, and the like. When combined with any mesh, fiber, and supporting layers of the strip, these materials generally affect the spreading of the blood on the strip. Reagent formulations must also adhere adequately to the strip and be uniform in order to give reasonable precision and accuracy. The total production cost of the strip must be low since it is used only once. The approximate chemical and biological reagent concentrations of a test strip are listed in the product information supplied by the manufacturer. Information for several brands of strips is shown in Table 1.

2.4. Hexokinase Method

The Bayer Glucometer Encore test strip is based on a variation of the hexokinase method in which hexokinase, ATP, and magnesium react with glucose to produce glucose-6-phosphate. The glucose-6-phosphate reacts with glucose-6-phosphate dehydrogenase and NAD^+ to produce NADH. The NADH then reacts with diaphorase and reduces the tetrazolium indicator to produce a brown compound (formazan). The brown color is directly proportional to the glucose concentration. The reaction sequence is as follows:



Hexokinase is available as a lyophilizate or a suspension in 3.2 M ammonium sulfate. The enzyme requires magnesium ions for catalytic activity. Other D-hexoses, such as fructose and mannose, are also phosphorylated by the enzyme but do not interfere with the assay. High-grade material has an activity greater than 450 U/mg at pH 7.6.

Table 1. Approximate Chemical and Biological Reagent Concentrations of Test Strips

Test Strip	Enzymes	Reagents	Other Ingredients
Encore	Microbial hexokinase (0.97%), microbial glucose-6-phosphate dehydrogenase (0.23%), microbial diaphorase (0.04%)	NAD (1.24%), ATP (3.56%), tetrazolium (3.68%)	Magnesium acetate (0.66%), potato lectin (0.01%), nonreactive (89.61%)
One Touch	Glucose oxidase (14 U/cm ²), peroxidase (11 U/cm ²)	3-Methyl-2-benzothiazolinone hydrazone hydrochloride (0.06 mg/cm ²), 3-dimethylaminobenzoic acid (0.125 mg/cm ²)	—
SureStep	Glucose oxidase from <i>Aspergillus</i> sp. (6.3 U), peroxidase from horseradish (5.6 U)	Naphthalenesulfonic acid salt (42 µg), 3-methyl 2-benzothiazolinone hydrazone, solubilized (27 µg)	—
Instant	Glucose oxidase (4.5 U)	Bis(2-hydroxyethyl)-(4-hydroximinocyclohexa-2,5-dienylidene)ammonium chloride (12.6 µg), 2,18-phosphomolybdate (80.0 µg)	Nonreactive and buffer (2.9 mg)
Easy	Glucose oxidase from <i>Aspergillus niger</i> (2.6%)	Potassium ferricyanide (9.5%), ferric sulfate 4-hydrate (2.1%)	Buffer (15.5%), nonreactive (70.3%)
Elite	Glucose oxidase (29.1%) from <i>A. niger</i> ; 20 U/mg	Potassium ferricyanide (32.0%)	Nonreactive (38.9%)
Advantage	Glucose oxidase (5.1%) from <i>A. niger</i>	Potassium ferricyanide (55.0%)	Buffer (20.9%), stabilizer (5.6%), nonreactive (13.4%)
ExacTech	Glucose oxidase from <i>A. niger</i> (3.0 U)	—	Nonreactive (0.1 mg)
Companion 2	Glucose oxidase from <i>A. niger</i> (0.02 U)	—	Nonreactive (60.0 µg)
Precision QID	Glucose oxidase from <i>A. niger</i> (0.02 U)	—	Nonreactive (60.0 µg)

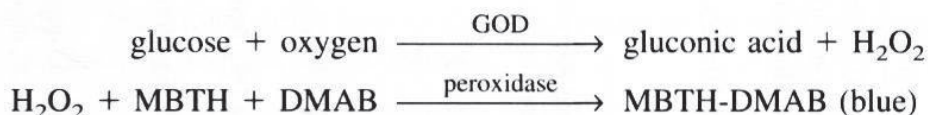
Glucose-6-phosphate dehydrogenase is available as a lyophilizate or a suspension in 3.2 *M* ammonium sulfate. For many materials the reaction rate with NAD^+ is about 1.8 times as fast as the same reaction with NADP^+ . Enzyme from some sources is selective for NAD^+ or NADP^+ . High-grade material has an activity greater than 550 U/mg at pH 7.6.

Diaphorase is available as a lyophilizate or a suspension in 3.2 *M* ammonium sulfate. Diaphorase from some sources reacts with NADH in preference to NADPH. High-grade material has an activity greater than 25 U/mg at pH 8.5. The enzyme molecule contains one molecule of flavin mononucleotide, the redox center.

The method is susceptible to disturbances due to endogenous and exogenous reducing substances, which can take the place of NADH. For example, high levels of ascorbate, bilirubin, and α -methyldopa can reduce tetrazolium compounds in the presence of an electron conductor such as diaphorase. Conversely, the enzyme is not specific for tetrazolium compounds, and a variety of dyes, especially dichlorophenol indophenol (DCPIP), can be reduced.

2.5. Glucose Oxidase–Peroxidase Optical Method

The LifeScan One Touch and SureStep test strips are based on a glucose oxidase–peroxidase–dye coupled system. When a drop of blood is applied, glucose oxidase catalyzes the oxidation of glucose in the blood by oxygen in the atmosphere and oxygen in the blood. Two new compounds, gluconic acid and hydrogen peroxide, are produced. The peroxidase then triggers the reaction of the hydrogen peroxide with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-dimethylaminobenzoic acid (DMAB). A naphthalenesulfonic acid salt replaces DMAB in the SureStep strip. The reaction sequence is



Glucose oxidase is most commonly available as a yellowish amorphous powder. Different grades may contain stabilizers as well as traces of polysaccharidases and catalase (which reacts with hydrogen peroxide to form water and oxygen). High-grade material has an activity greater than 250 U/mg. The enzyme has a molecular weight of about 160,000 and is approximately 74% protein, 16% neutral sugar, and 2% amino sugars. Glucose oxidase is composed of two identical polypeptide chain subunits covalently linked by disulfide bonds [19,20]. Each subunit molecule contains one molecule of iron and one molecule of FAD (flavine adenine dinucleotide). The crystal structure of partially deglycosylated glucose oxidase from *Aspergillus niger* has been determined [21]. The enzyme is highly specific for β -D-glucose, with a broad pH optimum from about 4 to 7. The

enzyme is inhibited by heavy-metal ions (Ag^+ , Hg^{2+} , and Cu^{2+}). Upon reaction with glucose, the FAD^+ redox centers are reduced to FAD. The reduced form of the enzyme can be oxidized by molecular oxygen or other electron acceptors (i.e., mediators such as organic dyes, nitrogen compounds, ferricyanide, or oxidized ferrocene derivatives; see the following sections). In each case, glucose is oxidized to gluconolactone. Gluconolactone is unstable in solution and hydrolyzes to produce gluconic acid.

Peroxidase is most commonly available as a reddish-brown powder. High-grade material has an activity of greater than 250 U/mg at pH 7. The enzyme contains one protohemin IX prosthetic group, with natural and amino sugars accounting for approximately 18% of the enzyme. Peroxidase is specific for the hydrogen acceptor; only peroxide, methyl peroxide, and ethyl peroxide are reactive. In contrast, the enzyme is not specific for the hydrogen donor. A large number of phenols, aminophenols, diamines, indophenols, leuco dyes, ascorbate, and several amino acids react [11].

The MBTH-DMAB dye couple was originally described for enzyme immunoassays [22]. Recent patents [23,24] claim that this indamine dye couple has the following advantages over traditional benzidine derivatives: (1) greater dynamic range, (2) improved enzymatic stability, and (3) noncarcinogenic. Stability and extended shelf life were claimed by using a concentrated buffer system (5 to 15 wt %) at a pH value near 4. A typical manufacturing technique is shown in Table 2.

The MBTH-DMAB couple also allows for correction of hematocrit and degree of oxygenation of blood with a single correction factor. At 700 nm both hematocrit and degree of oxygenation can be measured. The indamine dye chromophore absorbs at 635 nm but not significantly at 700 nm. A simplified version of the Kubelka-Monk equations has been applied to the 700-nm reflectance

Table 2. Glucose Oxidase-Peroxidase Optical Strip Manufacturing Technique

Step	Procedure
Dye solution	40 mg MBTH, 80 mg DMAB, 5 mL water
Initial dip	Dip a piece of Posidyne membrane (Pall Co.) into the dye solution, blot off excess liquid, and dry at 56°C for 15 min
Enzyme solution	6 mL water; 10 mg EDTA disodium salt; 200 mg Poly Pep, low viscosity (Sigma); 0.668 g sodium citrate; 0.523 g citric acid; 2.0 mL 6 wt% Gantrez AN-139 dissolved in water (GAF); 30 mg horseradish peroxidase, 100 U/mg; and 3.0 mL glucose oxidase, 2000 U/mL
Final dip	Dip the membrane into the enzyme solution, blot off excess liquid, and dry at 56°C for 15 min

reading to obtain a first-order correction for the 635-nm reading. A second-order correction to eliminate errors due to chromatography effects has also been developed [23,24].

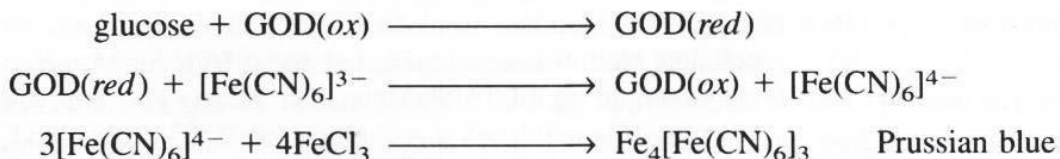
2.6. Glucose Oxidase–Organic Mediator Optical Method

The Boehringer Mannheim Corporation (BMC) Accu-Chek Instant test strip is based on a newly developed reductive chemistry [25]. Bis(2-hydroxyethyl)-(4-hydroximinocyclohexa-2,5-dienylidene)ammonium chloride is reduced by glucose to the corresponding hydroxylamine derivative and further to the corresponding diamine under the catalytic action of glucose oxidase. The diamine reacts with a 2,18-phosphomolybdic acid salt to form molybdenum blue. The reaction sequence is shown in Figure 2.

Recent patents [26–28] claim that a number of substances, including aromatic nitroso compounds and their tautomerically equivalent oxime compounds, are direct electron acceptors for the reduced form of glucose oxidase. Thus, depletion of oxygen, especially at high glucose levels, is avoided and the reaction endpoint may be reached very quickly. Hydrogen peroxide is not formed, so its side reactions are also avoided. Interestingly, sparingly soluble phosphomolybdic acid salts are stable at neutral and basic pH. At neutral pH the reaction with the diamine is apparently much faster than with other interfering reducing compounds. The absorption spectrum of molybdenum blue is relatively flat between 550 nm and more than 1100 nm, so the tolerance for variation in wavelength is very high. The molybdenum oxidation state is between 5 and 6 in the final product, molybdenum blue [28a]. A typical manufacturing technique is shown in Table 3.

2.7. Glucose Oxidase–Prussian Blue Method

The BMC Accu-Chek Easy test strip is based on the reaction of glucose oxidase with ferricyanide and the formation of potassium ferric ferrocyanide (Prussian blue). The reaction sequence is



Use of ferricyanide allows the reaction to proceed without oxygen. Thus, depletion of oxygen at high glucose levels and various factors affecting the oxygen partial pressure are avoided. Ferricyanide may also be more stable than organic dyes during drying steps in manufacture and storage through shelf life. A recent patent [29] claims that the choice of pH for the system is critical. Ferric salts

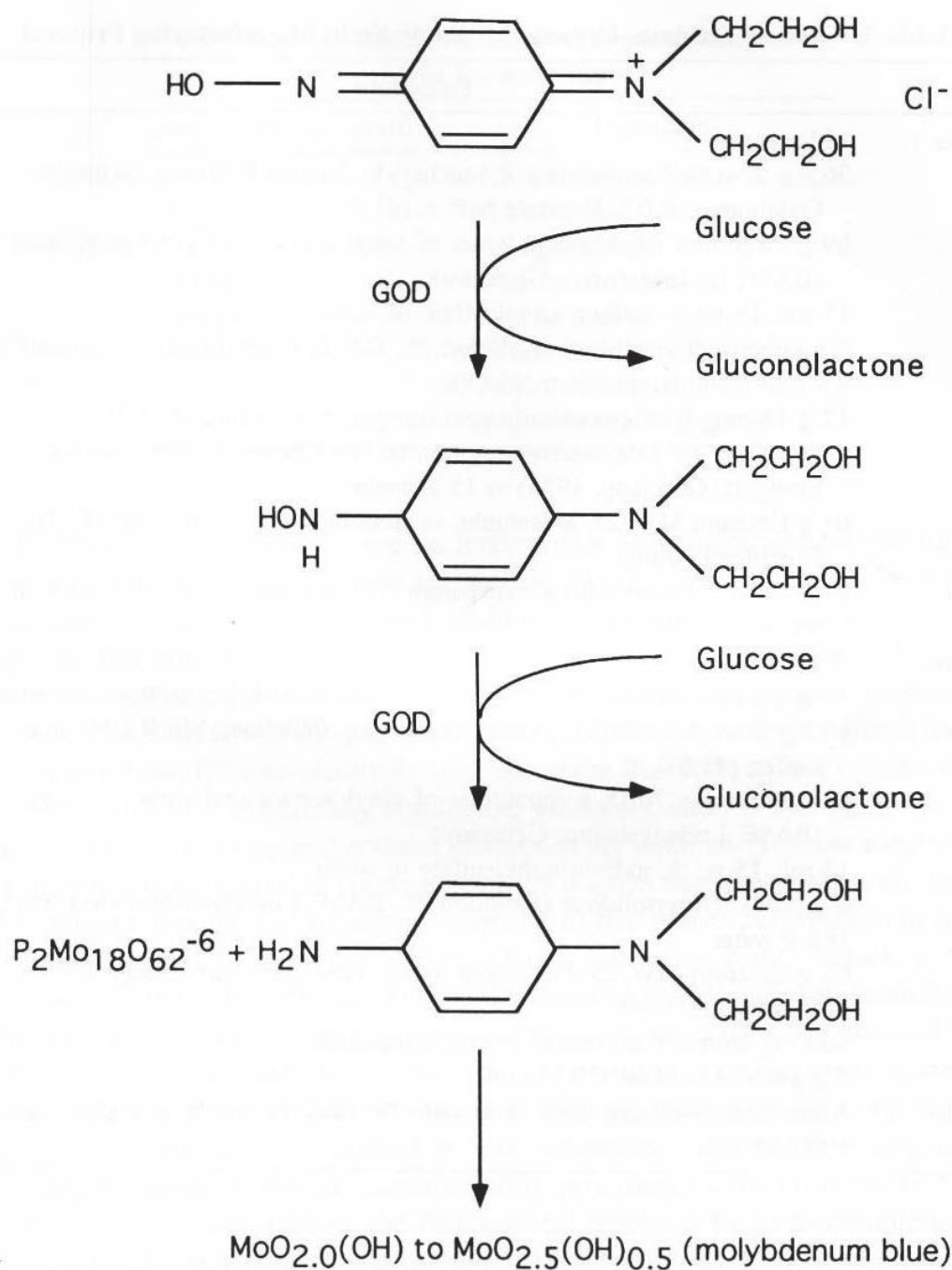


Figure 2. Reaction sequence resulting in the formation of molybdenum blue.

precipitate at high pH while glucose oxidase is denatured in acidic media. A pH of about 4 was preferred. A typical formulation is shown in Table 4.

2.8. Glucose Oxidase–Ferricyanide Electrochemical Method

The Bayer Glucometer Elite and BMC Accu-Chek Advantage test strips are single-use amperometric test strips. The reaction of glucose oxidase with glucose

Table 3. Glucose Oxidase–Organic Mediator Strip Manufacturing Protocol

Step	Procedure
Layer 1	113 g water 36.3 g 2 wt % Xanthan (a polysaccharide, Keltrol F, Kelco, Okmulgee, Oklahoma) in 0.2 M citrate buffer, pH 7 69 g Propiofan 70 D, a copolymer of vinyl acetate and vinyl propionate (BASF, Ludwigshafen, Germany) 15 mL 15 wt % sodium nonylsulfate in water 6 g polyvinylpyrrolidone (Kollidon 25, BASF, Ludwigshafen, Germany) 3.9 g tetrabutylammonium chloride 12 g 18-molybdodiphosphoric acid (prepared according to G. Brauer, <i>Handbuch der präparativen anorganischen Chemie</i> , F. Enke Verlag, Stuttgart, Germany, 1954) in 15 g water 63 g Celatom MW 25, kieselguhr, or diatomaceous earth (Eagle Picher, Cincinnati, Ohio)
Coat	Knife-coat 150 μm onto a transparent 200- μm -thick polycarbonate foil and dry at 60°C for 1 h
Layer 2	20 g water 16 g titanium dioxide RN 56 (Kronos-Titan GmbH, Leverkusen, Germany) 36.3 g 2 wt % Keltrol F (Kelco, Okmulgee, Oklahoma) in 0.2 M citrate buffer, pH 6.0 69 g Propiofan 70 D, a copolymer of vinyl acetate and vinyl propionate (BASF, Ludwigshafen, Germany) 15 mL 15 wt % sodium nonylsulfate in water 6 g polyvinylpyrrolidone (Kollidon 25, BASF, Ludwigshafen, Germany) 188 g water 63 g Celatom MW 25, kieselguhr, or diatomaceous earth (Eagle Picher, Cincinnati, Ohio) 400 mg aromatic nitroso or oxime compound 2 g glucose oxidase (200 U/mL)
Coat	Knife-coat a 400- μm -thick layer onto the first reagent layer and dry at 60°C for 1 h

and ferricyanide produces ferrocyanide, which is oxidized to produce an easily measured current. The reaction sequence is

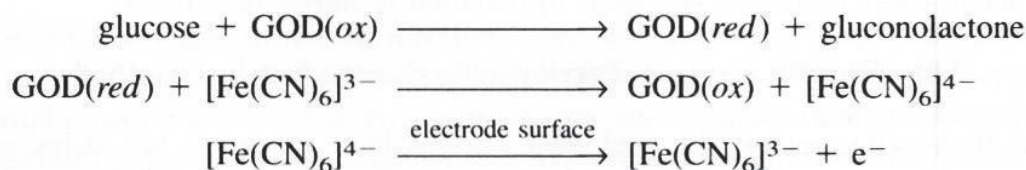


Table 4. Glucose Oxidase-Prussian Blue Strip Formulation

Amount per Kilogram	Ingredient
30 g	4-Aminobutyric acid
3.9 g	Ferric sulfate
36 g	Ferricyanide salt
1000 kU	Glucose oxidase
Remainder	TiO ₂ as a white pigment
	Tween-20 (nonionic surfactant)
	Propiofan-70D (film former)
	Natrosol (swelling agent)

Electrochemical sensors based on ferricyanide as an electron acceptor for the reduced form of glucose oxidase are well known [30,31]. These papers do not address the problem of ferrocyanide oxidation by oxygen. A recent patent [32] suggests that biosensors can be placed in the following categories: (1) three-electrode systems where a working electrode is referenced against a reference electrode (such as silver/silver chloride) and a counter electrode provides a means for current flow, (2) two-electrode systems where the working and counter electrodes are different electrically conducting materials, and (3) two-electrode systems where the working and counter electrodes are made of the same electrically conducting materials but the counter electrode is larger than the working electrode. This patent covering the Advantage strip claims that conventional wisdom in the electrochemical arts does not suggest that a biosensor could include a two-electrode system where the working and counter electrodes are substantially the same size (or the counter electrode is smaller) and made of the same material. The Advantage strip contains two palladium strips with an exposed surface area of about 6 mm² each and a sufficient amount of ferricyanide that the current produced during electrooxidation is limited by the concentration of ferrocyanide at the working electrode. A typical manufacturing procedure is shown in Table 5.

Typically, the strips allow the biochemical reactions to go to completion in about 40 s. Then a potential difference of 300 mV is applied across the electrodes. At this sufficiently high potential, ferrocyanide is rapidly oxidized when it has diffused to the working electrode. This diffusion-limited current is proportional to the concentration of ferrocyanide, which in turn is proportional to the glucose concentration.

2.9. Glucose Oxidase-Ferrocene Electrochemical Method

The MediSense ExacTech, Companion 2, and Precision QID test strips are single-use amperometric test strips. The reaction of glucose oxidase with glucose

Table 5. Enzyme Electrode Manufacturing Procedure

Step	Procedure
Prep 1	Stir 1.2 g Natrosol-250 (microcrystalline hydroxyethylcellulose available from Aqualon) in 1 L 0.74 M potassium phosphate buffer, pH 6.25. Allow to swell for 3 h.
Prep 2	Stir 14 g Avicel RC-591F (a microcrystalline cellulose available from FMC Corp.) in 505 mL water for 20 min.
Prep 3	Add 0.5 g Triton X-100 to 515 mL of Prep 1 and stir 15 min.
Prep 4	While stirring, add Prep 3 dropwise to Prep 2. Stir overnight.
Prep 5	Add 99 g of potassium ferricyanide a little at a time to Prep 4 while stirring. Continue stirring for 20 min. Adjust the pH to 6.25 by addition of potassium hydroxide.
Prep 6	Add 9.15 g glucose oxidase (219 U/mg from Biozyme) to Prep 5 and stir at least 20 min.
Prep 7	Add 20 g potassium glutamate to Prep 6 and stir at least 20 min. Filter through a 100- μ m sieve bag to remove any Avicel clumping.
Apply to strip	Add 6 μ L of Prep 7 to a 6 mm $\times$ 4 mm area of the test strip including two 1.5 mm $\times$ 4 mm exposed Pd electrodes. Dry by heating at 50°C for about 3 min.

and ferricinium ion produces ferrocene, which is oxidized to produce an easily measured current. The reaction sequence is shown in Figure 3. Ferrocene-based mediators were identified as having the well-behaved electrochemistry of ferro- and ferricyanide with the solubility and oxidation potential variations available through structural modification of the organic dyes. The second-order homogeneous rate constants for the reaction between ferricinium ion derivatives and the reduced form of glucose oxidase are large (0.26 to 5.25×10^5 L/mol·s, pH 7.0, $I = 0.1$) [33]. Interfering substances such as ascorbate, uric acid, cysteine, reduced glutathione, sodium formate, D-xylose, galactose, and mannose did not cause any interference either through direct electrode oxidation, reaction with the mediator, or inhibition of the enzyme [33]. A suspension formed as a printable and conductive ink has been described as useful for electrode construction [34]. The electrode can be manufactured by screen printing techniques in a multistep procedure comprising (1) screen printing of a Ag/AgCl reference electrode and metal tracing; (2) screen printing of the active electrode with a printing ink comprising a colloidal carbon, glucose oxidase in buffer, and an organic polymer; and (3) screen printing, spraying, or dip coating to provide a membrane over the assembly. Ferrocene or a ferrocene derivative is present in the ink.

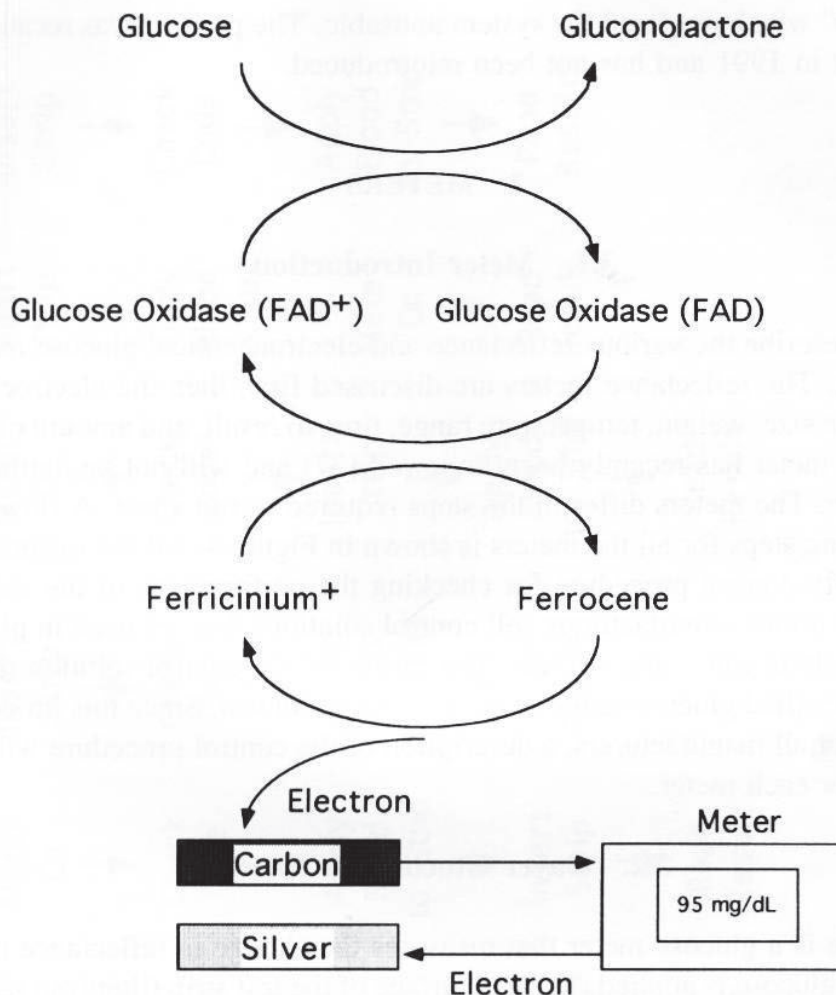


Figure 3. Electron transfer reactions with an electrochemical test strip.

2.10. Reusable Sensors

A reusable portable glucose sensor, the Eli Lilly/Elco Direct 30/30, was introduced to the U.S. marketplace in 1989 [35,36]. Every 30 days the user was prompted to replace the 1-in² glucose oxidase membrane positioned over a three-electrode system. In everyday use, the user was prompted to blot dry the sensor and apply a capillary blood sample to receive the result in about 30 s. A solution was provided for both cleaning and maintaining hydration of the sensor membrane. With proper care a frequent tester was able to realize significant cost savings on a monthly basis. Some users would not dry the sensor thoroughly prior to use, producing inaccurate results. Sometimes users did not clean the sensors properly after use. In addition, many users experienced a software

“freeze-up” which rendered the system unusable. The product was recalled from the market in 1991 and has not been reintroduced.

3. METERS

3.1. Meter Introduction

Next we describe the various reflectance and electrochemical glucose meters on the market. The reflectance meters are discussed first, then the electrochemical meters. The size, weight, temperature range, time to result, and amount of memory of each meter has recently been reviewed [37] and will not be duplicated in this chapter. The meters differ in the steps required to run a test. A flowchart of the operating steps for all the meters is shown in Figure 4. All the meters use the same quality control procedure for checking the performance of the meter and strip. The various manufacturers sell control solutions that are used in place of a blood sample to run a glucose test. The results of the control solution must fall within a specified glucose range to have a successful test. Since this procedure is the same for all manufacturers, a description of the control procedure will not be repeated for each meter.

3.2. Bayer Glucometer Encore

The Encore is a glucose meter that measures the change in reflectance of a test strip when glucose is applied. The top surface of the test strip filters out red blood cells with the aid of an agglutination reaction. The glucose in the plasma portion of the sample reacts in a series of reactions with hexokinase, ATP, magnesium, glucose-6-phosphate dehydrogenase, NAD^+ , diaphorase, and tetrazolium to produce the brown-colored formazan (see Section 2.4). The change in reflectance due to the formation of the formazan is measured.

The user starts a test by turning the meter on. The user checks that the meter calibration (program number) matches their test strips. The user then opens the testing compartment. A test strip is removed from its individual foil pouch. The user lances a finger and applies a drop of blood to the test cup on the test strip. The user then inserts the strip into the meter within 30 s after applying the blood to the strip. Results are reported in 20 to 60 s. Each box of strips has an assigned program number that the user can select from the meter. The program number provides the calibration data for that lot of strips. The performance of the optics is checked with a check paddle that is inserted like a test strip. The meter reads the check paddle like a real test strip. The user compares the check paddle result against an expected range. The strip guide that the test strips are inserted into is removable so that it can be washed.

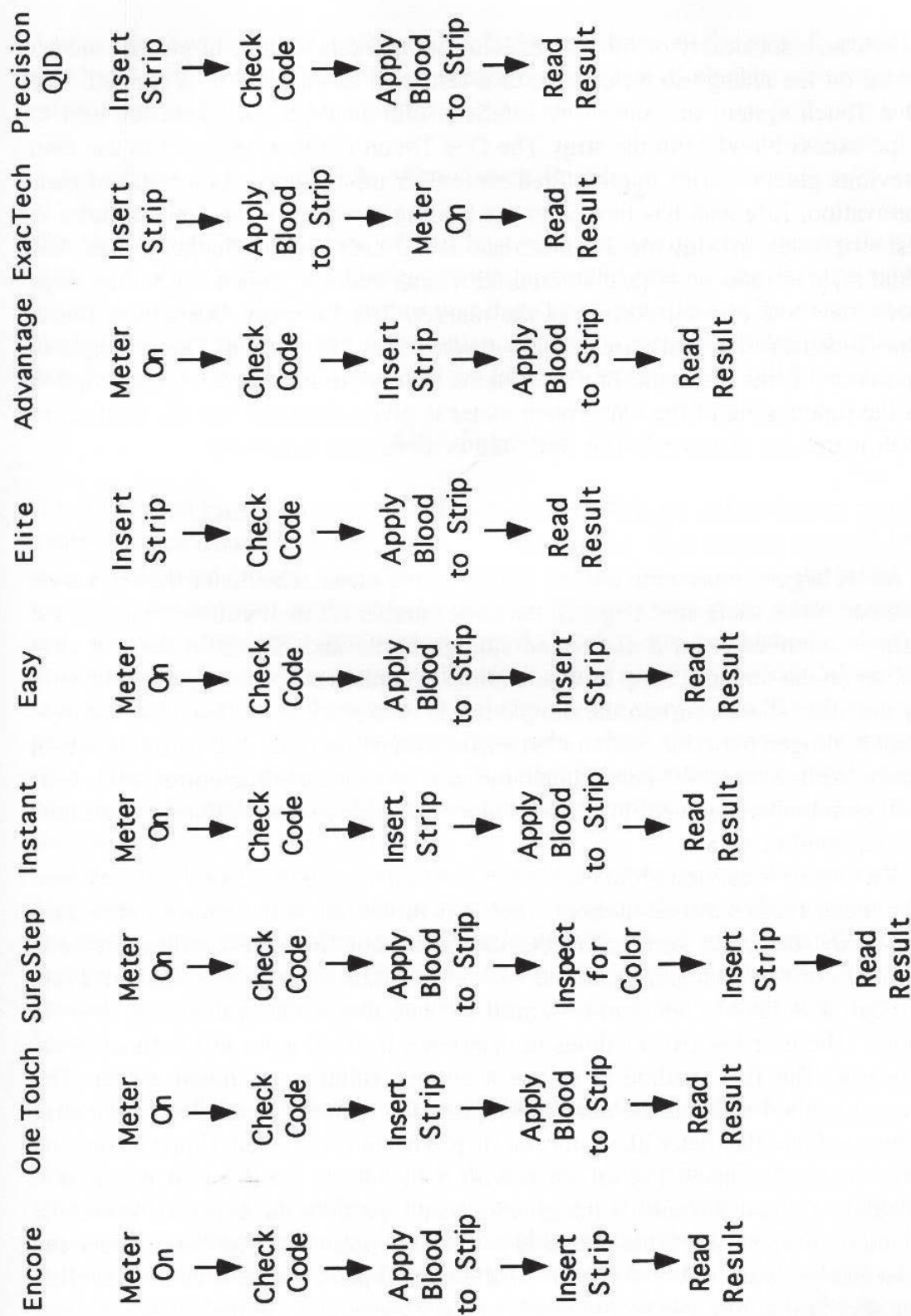


Figure 4. Flowchart of glucose testing.

3.3. LifeScan One Touch

LifeScan, a subsidiary of Johnson & Johnson, markets a series of glucose meters based on the change in reflectance of a test strip when glucose is applied. The One Touch system introduced by LifeScan eliminated the need for the user to wipe excess blood from the strip. The One Touch strips were easier to use than previous glucose strips that required more user interactions. As a result of their innovation, LifeScan has moved to the leading position in the United States in test strip sales. Worldwide, LifeScan and BMC have similar market shares. The older style of reagent strips that required wiping and user-dependent timing steps now constitute a small portion of the market. The LifeScan One Touch Basic, One Touch Profile, and SureStep are described in this section. The principle of operation of the Basic and Profile systems is very similar. A general description of the functioning of the One Touch meter is given, then the specific features of each meter are discussed. The SureStep is discussed separately.

3.4. LifeScan Basic and Profile Operation

A test is begun by pressing the ON button on the meter. The meter flashes a code number which users check against the code number for their current strips. A test strip is removed from a desiccated storage bottle and placed in the test strip holder on the meter. A drop of whole blood is applied to the test spot on the strip by the user. The glucose in the sample reacts with glucose oxidase in the strip to form hydrogen peroxide, which then reacts with peroxidase. The peroxidase then reacts with 3-methyl-2-benzothiazolinone hydrazone hydrochloride and 3-dimethylaminobenzoic acid to form a colored complex (see Section 2.5). Results are reported in 45 s.

The meter is calibrated for each lot of test strips through use of a code number. The meter flashes a code number when it is turned on. If the code number does not match the code number on the user's current lot of test strips, the code number must be changed. A button labeled C on the meter allows users to cycle through a series of code numbers until the one that matches their test strips is found. The user has two methods to determine if the glucose test is functioning properly. The first method is to use a control solution, as noted earlier. The second method is to use the check strip provided with the meter. The check strip is inserted into the meter like a normal strip. Then it is removed, flipped over, and reinserted in the meter. The reflectance off both sides of the check strip is used to calculate a glucose result. If the glucose result matches the expected value, the meter, more specifically the optics, is performing properly. If an error occurs, the most likely cause is contamination of the optical path with particulate material. The test strip holder can be removed from the meter and cleaned, as can the test area underneath the test strip holder, which contains an optical window.

3.5. One Touch Basic

As the name implies, the One Touch Basic does not have all the extra features of the One Touch Profile. The meter can recall only the last result. The meter can be set to operate in English or Spanish.

3.6. One Touch Profile

The One Touch Profile is one of the most sophisticated glucose meters on the market. The meter has the capacity to store up to 250 results. The user can input information about when the testing was done (e.g., prior to lunch or after exercise). The user can also input information about the type and number of units of their last insulin injection. The meter can be set to report results in 19 languages.

3.7. SureStep

A test is begun by pressing the ON button on the meter. The meter flashes a code number which users check against the code number for their current strips. A test strip is removed from a desiccated storage bottle. A drop of whole blood is applied to the test spot on the strip by the user. The user then waits a few seconds and checks for formation of a blue spot on the back of the strip, which indicates that the strip is functioning properly. Within 2 min of applying the blood drop, the user places the strip into the meter. The glucose in the sample reacts with glucose oxidase in the strip to form hydrogen peroxide, which then reacts with peroxidase. The peroxidase then reacts with 3-methyl-2-benzothiazolinone hydrazone and naphthalenesulfonic acid to form a colored complex (see Section 2.5). Results are reported on average in 30 s.

The meter is calibrated for each lot of test strips through use of a code number. The meter flashes a code number when it is turned on. If the code number does not match the code number on the user's current lot of test strips, the code number must be changed. A special button on the meter allows users to cycle through a series of code numbers until the one that matches their test strips is found.

3.8. Boehringer Mannheim Accu-Chek Instant

The Instant is a glucose meter that measures the change in reflectance of a test strip when glucose is applied. The active area of the strip contains glucose oxidase, a quinonediimine oxide, and phosphorous molybdic acid salt. With the quinonediimine acting as a mediator, the phosphorous molybdic acid is reduced to molybdenum blue (see Section 2.6). The change in reflectance due to the formation of molybdenum blue is read by the meter.

A test is started by turning the meter on. The user then obtains a test strip from the test strip bottle. Users check that the meter calibration (code number) matches their test strips. A test strip is inserted into the meter. The user applies a drop of blood to the strip. Results are reported in 12 s. A qualitative check of the result can be made by comparing the color generated on the strip to a color scale on the test strip bottle. Each vial of test strips has an assigned code number that the user can select from the meter. The code number provides the calibration data for that lot of strips. Because bloody strips must be inserted into the instrument to be read, the manual suggests cleaning the test strip guide once per vial of 50 test strips. The Instant is designed for use on capillary blood and is not recommended for venous samples.

3.9. Boehringer Mannheim Accu-Chek Easy

The Easy is a glucose meter that measures the change in reflectance of a test strip when glucose is applied. The active area of the strip contains glucose oxidase, potassium ferricyanide, and ferric sulfate. When blood is added to the strip, glucose reacts with glucose oxidase to cause the oxidation of glucose and the reduction of glucose oxidase. The reduced glucose oxidase is oxidized by ferricyanide, which causes the reduction of ferricyanide to ferrocyanide. The ferrocyanide reacts with ferric ion to produce the colored species, Prussian blue (see Section 2.7). The change in reflectance due to the formation of Prussian blue is measured and is proportional to the amount of glucose present.

A test is started by turning the meter on. Users check that the meter calibration (code number) matches their test strips. The user then obtains a test strip from the test strip bottle. The diabetic lances a finger and applies a drop of blood to the test strip. The user then waits 5 to 30 s before inserting the strip into the meter. Results are reported in 15 to 60 s. A qualitative check of the result can be made by comparing the color generated on the strip to a color scale on the test strip bottle. Each bottle of test strips comes with an Easy Key that is inserted into the instrument. The Easy Key contains calibration information for that specific lot of test strips. The Easy is capable of storing up to 350 results in its memory, and it can store any of 14 event codes with the result. A meaning is assigned to each event code by the user. Because bloody strips must be inserted into the instrument to be read, the manual suggests cleaning the test strip guide once per vial of 50 test strips. The Easy is designed for use on capillary blood and is not recommended for venous samples.

3.10. Bayer Elite

The Elite glucose test strip is manufactured in Japan by Matsushita and Kyoto Daiichi Kagaku. The Elite test strip is sold in the United States by Bayer,

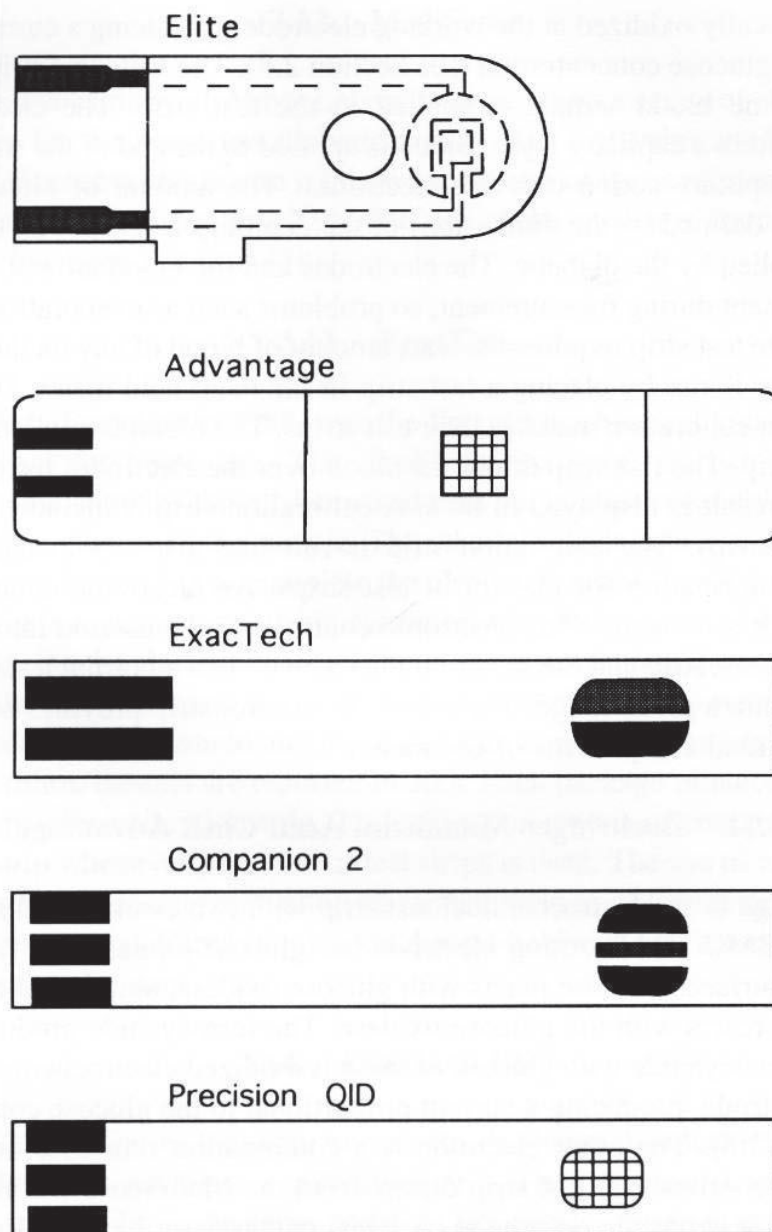


Figure 5. Electrochemical glucose test strips. Elite electrodes shown in phantom are visible but inside the strip. Test strips are not drawn to scale.

formerly Ames. It is an electrochemical test strip with two electrodes and is manufactured using screen printing techniques. A square inner working electrode is almost completely surrounded by a circular electrode that acts as both a reference and counter electrode, as shown in Figure 5. Glucose oxidase and ferricyanide are dried on the working electrode. Glucose reacts with glucose oxidase and then the mediator, ferricyanide, reacts with the glucose oxidase. The ferrocyanide produced by the reaction of ferricyanide with glucose oxidase is

electrochemically oxidized at the working electrode, producing a current proportional to the glucose concentration (see Section 2.8). The Elite test strip is unique in the way the blood sample is applied to the test strip. The electrodes are contained within a capillary layer. Blood is applied to the end of the test strip and moves by capillary action over the electrodes. The amount of blood over the electrodes is defined by the dimensions of the capillary layer, not by the amount of blood applied by the diabetic. The electrodes and the blood are not exposed to the environment during measurement, so problems such as evaporation are minimal. The Elite test strip requires the least amount of blood of any on the market, 3 μL . An assay is run by placing a test strip in the hand-held meter. Users check that the meter calibration matches their test strips. The blood is applied to the end of the test strip. The test strip draws the blood over the electrodes using capillary action. The result is displayed in 60 s. A calibration strip is included with each box of test strips. The calibration strip is run like a test strip and provides calibration information for that lot of test strips. An electronic control strip is provided with each meter. The electronic control strip is inserted into the meter like a normal test strip and the meter outputs a result like a normal test. The result must fall within a given range. The electronic control strip provides a test of the internal electrical components of the meter.

3.11. Boehringer Mannheim Accu-Chek Advantage

The Advantage is an electrochemical test strip with two electrodes that is manufactured by BMC. The working electrode has glucose oxidase and ferricyanide dried on its surface. Glucose reacts with glucose oxidase, and then the mediator, ferricyanide, reacts with the glucose oxidase. The ferrocyanide produced by the reaction of ferricyanide with glucose oxidase is oxidized electrochemically at the working electrode, producing a current proportional to the glucose concentration (see Section 2.8). The other electrode is a combination reference and counter electrode. The Advantage test strip differs from the MediSense and Bayer electrodes in that it is not screen printed. A BMC patent describes metal stripes that are laminated to a support [32]. The test strip (shown in Figure 5) is substantially larger than the MediSense Precision QID and Bayer Elite electrodes, leading to larger sample volume requirements. The electrodes are exposed, so the diabetic applies the blood directly to the electrodes. The Advantage requires a minimum of 13 μL of blood. Users run the glucose test by turning the meter on and checking that the meter calibration matches their test strips. A test strip is inserted into the meter. The user then places a drop of blood on the strip. Results are reported in 40 s. A calibration chip is shipped with each box of test strips. The chip is placed into a slot on the meter and provides the calibration data for that lot of test strips. The meter is a small hand-held device. The Advantage is designed for use on capillary blood and is not recommended for venous samples.

3.12. MediSense

MediSense, a subsidiary of Abbot Laboratories, markets a series of electrochemical test strip for the home use diabetic market. The test strips are based on the reaction of glucose with glucose oxidase and the use of the mediator ferrocene to cycle the enzyme (see Section 2.9). The use of the mediator ferrocene and its derivatives has been patented [38].

3.13. ExacTech

The ExacTech, launched in 1988, was the first electrochemical glucose test strip on the market for home use. The ExacTech test strip has two symmetrical electrodes. One contains the mediator and enzyme in a water-soluble matrix and reacts with the glucose in the sample. The other electrode acts as a reference and counter electrode, serving to complete the electrochemical circuit (see Figure 5). The test strip is manufactured using screen printing techniques. The glucose test is run by placing the electrical contact end of the test strip into the meter. Blood is applied to the electrodes and the meter button is then pressed to start the test. The current produced is measured and translated into a glucose measurement using a stored algorithm. Results are reported in 30 s. Each package of electrodes comes with a lot-specific calibration strip. The calibration strip is inserted into the meter like a test strip whenever a new lot of test strips is used. The use of electrochemical versus reflectance detection allowed miniaturization of the meter not possible before. The meter is offered in a standard hand-held size and a meter that is the size and shape of a pen.

3.14. ExacTech RSG

The ExacTech RSG uses the same two-electrode electrochemical test strip as the ExacTech but does not have a calibration strip. The RSG test strips are factory calibrated, so each lot is the same and does not require the user to do a calibration. The RSG also has a sensor check strip, which can be inserted into the meter like a test strip. The sensor check strip provides a result that indicates whether the electrical components of the meter are functioning properly.

3.15. Companion 2

The Companion 2 series of test strips added a third electrode to the test strip. Two symmetrical electrodes are separated by a thin center electrode (see Figure 5). One of the two symmetrical electrodes is a working electrode which contains glucose oxidase and a ferrocene mediator. The other symmetrical electrode does not contain glucose oxidase, so it does not react with glucose. It will, however,

react with any electrochemically active components in the blood (see Section 4.4). The current from the dummy electrode is subtracted from the working electrode to yield a net current due only to glucose. The middle electrode acts as a combination reference and counter electrode in a manner similar to the Exac-Tech. The glucose test is run by inserting the test strip into the meter, which activates the meter. Blood is applied to the electrodes. The current flowing between the electrodes activates the timing sequence of the measurement. This eliminates the need to press a start button. Results are reported in 20 s. Each package of electrodes comes with a lot-specific calibration strip. The calibration strip is inserted into the meter like a test strip whenever a new lot of test strips is used.

3.16. Precision QID

The Precision QID uses the three-electrode technology developed for the Companion 2 test strip. The electrodes are not visible in the Precision QID strips. Blood is applied to a target area away from the electrodes and wicks over the electrodes (see Figure 5). The advantages of such a test strip design are many. The diabetic no longer controls the amount of blood that is over the electrodes. The amount of blood is controlled by the geometry of the flow path above the electrodes. The blood over the electrodes is not exposed to the environment during the measurement. This eliminates problems such as evaporation of the sample during the measurement. The Precision QID is the only test strip on the market that combines three-electrode measurement for reduction of interference's with the isolation of the measurement from the environment. The Precision QID strip was designed so that it could be used in Companion 2 meters (backward compatible). The glucose test is run in a similar manner to the Companion 2 except that calibration information is supplied at the start of the Precision QID test.

4. PERFORMANCE

4.1. Performance Introduction

The glucose biosensor is the only quantitative clinical assay that is done by the general public in their homes. Pregnancy tests are performed at home but provide only yes or no results. Recently, non-biosensor-based assays for cholesterol have been introduced for the general public's use. The cholesterol assays are not instrument based. The unique aspects of the glucose assay are that it is run by nonclinical personnel in a nonclinical setting. This presents performance issues that are not normally encountered in clinical chemistry. The glucose assay also

has all the normal performance issues associated with a clinical assay. The difficulty of achieving good performance from a home glucose test was recently highlighted by a study done in New Zealand. New Zealand's drug reimbursement agency commissioned a study that tested the 13 glucose meters available there and found that none of them met the American Diabetes Association guidelines for performance [39]. Their conclusion is both troubling and believable. In this section we explain why all the meters could have failed.

As highlighted in this section, the performance issues associated with home glucose testing are many. In this section we describe how the accuracy of home glucose meters is measured and the accuracy differences between meters. The precision of the glucose assay is discussed in a similar manner. The sources of performance problems associated with blood chemistry, diabetic physiology, user interactions, and environmental conditions are described. The overall performance of many of the glucose meters has been rated by *Consumers Reports* [40].

4.2. Accuracy

The glucose assay can be treated like any other clinical assay when the accuracy is measured. The various manufacturers measure samples on their instrument and a clinically accepted reference instrument. A commonly used reference instrument is the Yellow Springs Instruments, Inc. (YSI) glucose analyzer. The YSI analyzer performs a dilution of the blood sample and uses glucose oxidase to react with glucose in the sample. Hydrogen peroxide is produced, which is oxidized electrochemically at a platinum electrode. Proprietary selective membranes are used to block electrochemically active substances in the blood, such as uric acid, ascorbic acid, and acetaminophen. The blood sample is diluted to eliminate the problem of too little oxygen in blood to react with glucose and glucose oxidase at high glucose concentrations. The dilution brings the glucose concentration down into the range where the reaction is not oxygen limited. The dilution does not eliminate the problems experienced by glucose meters with high and low hematocrit levels in blood [41]. The performance of the YSI analyzer is typical for a clinical chemistry assay. One drawback to using a YSI glucose analyzer is that it requires 25 μL of blood to make a measurement. This volume is more than twice as much as is required for a typical glucose meter. Obtaining 25 μL requires extensive milking of the finger, which is unpleasant for the user.

A bias exists between whole blood and serum or plasma. The difference is due to the red blood cells in whole blood. The concentration of glucose in the extracellular space of blood, plasma, is approximately the same as that in the intracellular space of the red blood cells. The red blood cells are actively metabolizing glucose, but the transport of glucose into the cells is fast enough to maintain equilibrium. The difference in glucose levels between whole blood and

Table 6. Meter Performance^a

Meter	Correlation	Ascorbic Acid ^b	Triglyceride (mg/dL)	Sodium Fluoride
Encore	Plasma	Normal	1000	Yes
One Touch	Whole blood	Normal	3000	No
SureStep	Plasma	3 mg/dL	3000	No
Instant	Whole blood	—	375	—
Easy	Whole blood	Therapeutic	7000	—
Elite	Plasma	Normal	3000	No
Advantage	Whole blood	—	2000	—
ExacTech	Whole blood	3 mg/dL	—	No
Precision QID	Whole blood	3 mg/dL	3000	No

^aValues are from manufacturers' user manuals, test strip inserts, or technical support unless otherwise noted.

^bAscorbic acid normal range, 0.4–2.0 mg/dL [43].

plasma or serum is due to the volume of blood occupied by the red cell membrane. In a person with a normal hematocrit, the cell membrane occupies approximately 11% of the volume of blood. The cell membrane contains a low level of water. The reaction of the biosensors typically depends on the amount of glucose per volume. Whole blood then typically reads 11% lower than serum or plasma because of the red blood cell membrane. All glucose biosensor manufacturers must choose whether they want their instruments to output a result that correlates to the glucose concentration in the whole-blood sample or to apply a correction factor to read out the result that would be expected in plasma. The decision that each manufacturer must make about which result to report is not easy. Many of the problems associated with the accuracy of glucose meters center around this correlation issue. The trend among the most recently introduced meters is to correlate to plasma. The meters and what they are correlated to is listed in Table 6. The trend to plasma correlation is probably caused by the way the user and physician perceive the meter's performance. If users want to check a meter's accuracy, they will go to their physician and compare their glucose meter's result to a venipuncture sample taken at the physician's office. Since the venipuncture sample will typically be run as a plasma or serum sample by the physician's office, a plasma result offers the best perceived accuracy. Some of the meter manuals explain how to apply the plasma to whole-blood conversion. How much of this is understood by the user and physician is not clear. What has resulted is the rule of thumb that glucose results are acceptable if they are within 20% of the laboratory value [39,42]. The sources of inaccuracy are discussed in this section.

Manufacturers will typically run a correlation of their meters' results versus a standard clinical instrument, such as a YSI glucose analyzer. The manufacturers

then report slope, intercept, and correlation coefficient from the correlation. The correlation among the manufacturers reported in their test inserts is close and will not be reported here. As would be expected, if a good correlation could not be obtained, a manufacturer would not put the meter on the market. The performance of the Encore [44,45], Easy [46,47], Elite [44,48], Advantage [49], and Precision QID [50] have been reported.

A different method of looking at the glucose correlation was proposed by Clarke in 1985 [51,52]. The meter is correlated with a glucose analyzer as usual. The difference is in the interpretation of the correlation plot. Clarke defined different zones of the plot, depending on their clinical importance. The Clarke error grid is shown in Figure 6. If the glucose meter result is within 20% of the reference instrument or both are below 70 mg/dL, that point falls into zone A. Correlation points within zone A are considered to be clinically correct because they would lead to correct treatment decisions. Glucose meter results greater than 20% from the reference instrument but which would lead to no adverse treatment by the patient fall into zone B. If the diabetic's glucose is in the normal range but the glucose meter reads it outside that range, that would fall into zone C. Results falling into zone C might lead diabetics to treat themselves when it would not be

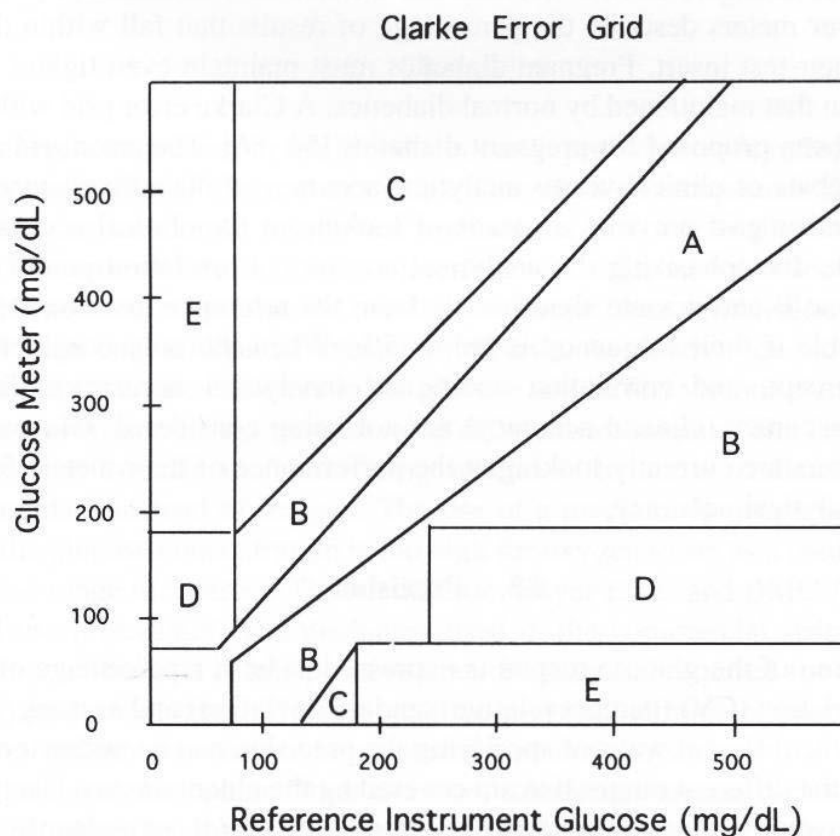


Figure 6. Clarke error grid.

necessary, because their glucose is normal. If the glucose meter reads a diabetic's glucose as being normal when it is actually abnormal, that point would fall into zone D. In zone D diabetics fail to treat themselves even though the glucose is actually abnormal. If the diabetic's glucose is high but the glucose meter reads it as being low, or vice versa, that point falls in zone E. In zone E diabetics would give themselves the opposite treatment from that required, possibly causing severe health problems.

An example of a point in zone C is a diabetic with a normal glucose of 140 mg/dL with a meter reading of 50 mg/dL. The person would normally eat something to elevate what is believed to be a low glucose. The result would be that the diabetic would elevate the glucose level out of the normal range but not into a range that would compromise the person's immediate health. If the opposite situation occurred, it could have an immediate impact on the person's health. A glucose level of 50 mg/dL that the meter read as 140 mg/dL would fall into zone D. The user would not know that his or her glucose is low and in need of immediate action. The Clarke error grid provides information about the performance of glucose meter that would not be available from a standard correlation plot. Errors at certain zones of the correlation plot are more damaging to a diabetic than in other zones. The acceptable limit for meter performance is 95% of the values in zone A, 5% in zone B, and 0% in zones C, D, and E [53]. Some of the newer meters describe the percentage of results that fall within different zones in their test insert. Pregnant diabetics must maintain even tighter glucose control than that maintained by normal diabetics. A Clarke error grid with tighter limits has been proposed for pregnant diabetics [54–56]. The error grid analysis started a debate of clinical versus analytical accuracy of diabetic glucose testing [53]. The error grid provides a means of looking at the clinical accuracy of a result while deemphasizing the analytical accuracy. Correlation points can fall into the zone B and deviate significantly from the reference instrument but still be acceptable if their frequency is below 5%. When the only consideration is slope, intercept, and correlation coefficient (analytical accuracy), diabetics' needs for accuracy (clinical accuracy) are not being considered. Glucose meter manufacturers are currently looking at the performance of their meters for clinical and analytical accuracy.

4.3. Precision

The precision of the glucose meters is expressed as both a percentage of coefficient of variation (CV) (percent relative standard deviation) and as $\pm$ mg/dL. The reason for the different ways of specifying the precision can be understood when we look at the different ranges that are covered by the glucose assay. Using a goal of 5% CV would require a standard deviation of 15 mg/dL at a glucose level of 300 mg/dL but a standard deviation of 2.5 mg/dL at a glucose level of 50 mg/dL.

The Clark error grid described in section 4.2 offers insight into what an acceptable precision would be (see Figure 6). A 5% CV at 300 mg/dL would mean that 95% of the results would fall within ± 30 mg/dL, 2 standard deviations. Comparing this level of precision to the Clark error grid would mean that the results would fall within the acceptable zone A of the grid. A 5% CV at 50 mg/dL would mean that 95% of the results would fall within ± 5 mg/dL. Comparing this to the grid would mean the results are well within zone A. In fact, the precision could be much worse at 50 mg/dL and still fall within zone A. The allowance for less precision at lower glucose levels is fortuitous. In terms of % CV, both reflectance and electrochemical glucose meters lose their precision at low glucose concentrations. As with most analytical techniques, noise becomes a problem at low signal levels. Sources of imprecision are discussed in the following sections.

A source of imprecision that is difficult to explain in detail is the variability within and between lots caused by manufacturing. Each manufacturer has different methods of manufacturing and for trying to control this variability. All glucose meters except the ExacTech RSG have a test-strip-lot-specific calibration to reduce the lot-to-lot variability. The name-brand glucose test strip manufacturers have been able to achieve better precision than have companies trying to make generic replacements for the name-brand test strips [57]. This indicates that manufacturing expertise does contribute to the precision of glucose test strips.

4.4. Chemistry

Various chemicals present in the blood can cause problems with reflectance and electrochemistry-based glucose test strips. One class of compounds that can be responsible for inaccurate results are compounds that can easily be oxidized [58,59]. The action of the oxidizable interferent is different for reflectance than for electrochemical strips, but the result is the same: inaccurate values. Electrochemical strips can oxidize the interferent directly. Any current due to oxidation of the interferent creates an error in the glucose result. The various electrochemical strips handle interfering species using different techniques. The first method used to lessen the effect of interfering species is to use a mediator to cycle glucose oxidase instead of oxygen. The use of a mediator is necessary anyway because the glucose concentration is too high for oxygen to act as a mediator over the glucose range of interest. The MediSense, Bayer Elite, and BMC Advantage strips all use a mediator. The mediators used in the commercial strips are oxidized at a potential that is less positive than the potential required for the oxidation of hydrogen peroxide. The less positive potential eliminates problems with interfering species that require more positive potentials, such as that caused by uric acid and acetaminophen. Other causes of interfering species such as ascorbic acid (see Table 6 for the acceptable level) and dopamine, are still a problem because they require less positive potentials to be oxidized. Both MediSense test

strips and Elite test strips use nonnoble metals as working electrodes. An organic oxidizable interferent is not readily oxidized at the carbon electrodes used in MediSense and Elite strips, providing more interference rejection than at a noble metal such that used in the BMC Advantage strip. The MediSense Precision QID strip is the only biosensor to incorporate a third electrode. Any oxidizable interfering species will react at the third or dummy electrode. The current from the oxidizable interference can then be subtracted from the current at the working electrode to correct for the interferent.

An oxidizable interferent can also interfere with chemical reactions producing colored complexes in the reflectance glucose strips. The oxidizable interferent can be oxidized instead of the dye that is supposed to be oxidized in the color formation step. An example would be oxidation of ascorbic acid by ferricyanide in the Accu-Chek Easy reaction scheme. The ferrocyanide produced would react with ferric ions to form Prussian blue (see Section 2.7). In reactions involving peroxidase, One Touch and SureStep, the peroxidase is oxidized by hydrogen peroxide produced by the reaction of glucose and glucose oxidase. The oxidized peroxidase oxidizes a dye to form a colored complex that is read by reflectance. Peroxidase can also oxidize ascorbic acid, uric acid, and bilirubin [60]. The oxidizable interferent that is oxidized instead of the dye then directly decreases the amount of colored complex formed and the resulting glucose concentration reported. The concentrations of ascorbic acid (see Table 6 for an acceptable level), uric acid, and acetaminophen are typically much lower than the glucose concentration. When the concentration of the oxidizable interferent is low, the error will not be significant. However, at elevated levels of the interferent the error can be significant.

Blood components that affect the optical properties of the blood can cause problems for reflectance meters. Most reflectance meters indicate a level of triglycerides that will not interfere with the assay (see Table 6). Above a certain level the triglyceride level can cause a problem. This will depend on the specific wavelengths being used for each type of strip. The electrochemical strips do not suffer from problems associated with colored components in the blood. The blood can become so thick that it does not effectively diffuse through the membranes of the reflectance strip. This can occur at very high protein levels. For this reason optical strips carry a warning about disease states that can cause such problems. This is typically not a problem for electrochemical strips.

Several meters cannot be used with blood drawn into tubes containing sodium fluoride as an anticoagulant (see Table 6). Meters that cannot run venous samples do not report any recommendation for the use of sodium fluoride. Fluoride is known to inhibit many enzyme reactions [61]. Commonly accepted anticoagulants are lithium and sodium heparin. Since home glucose testing is done exclusively with a fingerstick, this is not a problem in home testing, but hospitals would like to use whatever source of blood is available.

4.5. User Interactions

The biggest source of errors in home glucose testing involves interactions of the user with the glucose assay. This is not surprising because the user is not a trained laboratory technician. A common complication of diabetes is retinopathy, which leads to poor eyesight, which complicates the problem. The diabetic often receives some cursory training at a physician's office. However, with trends in health care leading away from patients seeing specialists, diabetics will be less likely in the future to see a specialist who could provide the necessary training. They are for the most part left on their own to discover the problems that may occur. Recently, manufacturers have been trying to address some of the user-related performance issues in home glucose testing with new products described as less prone to user error. The problems and these new approaches are explained in the following section.

Two consecutive steps in the testing process that sound very simple create most of the problems. The first critical step involves diabetics lancing a finger to obtain a blood sample for testing. Typically, a finger is lanced, but other well-vascularized areas of the body, such as the earlobe, can also be used. The amount of pain involved varies from person to person, and most diabetics describe it as more painful than the insulin injection. Lancing creates a small opening in the skin. Typical lancets have a diameter equivalent to that of a 24- to 30-gauge needle. The standard depth of penetration is 1.6 to 2.2 mm when fired from a typical lancet device. This creates a clean hole down through the upper layer of the skin into the capillary beds that feed the epidermis. Lancing itself will not usually generate the 3 to 15 μL of blood needed for glucose meters. The diabetic must then squeeze the hand or finger to force out enough blood for the assay. Manufacturers recommend warming the hand prior to lancing and putting the hand down at the side to improve the blood flow. Squeezing can cause soreness which can continue for several days. Because of the problems associated with obtaining blood, many diabetics will try to get just enough blood to run the test. They have no accurate way of knowing if they have enough blood for the test strip. Most manuals describe the correct amount of blood as enough to form a "hanging drop" of blood on the finger, a round dome of blood that forms when the finger is turned over. The drop has enough volume to appear to hang, as opposed to a smaller drop which will appear to conform to the shape of the finger. This crude measurement, along with the knowledge that a diabetic will gain after doing this hundreds of times, is the only way the diabetic can gauge the volume. This brings the diabetic to the second critical step, placing the blood drop on the sensor area of the test strip.

Most test strips try to clearly identify a target area on which the blood drop should be placed, to make it easier to use the test strip. Some strips use color to define that area and others have created a well into which the blood is dropped.

The Elite is unique because it does not have a target area. The edge of the Elite test strip is touched to the blood drop and the blood is pulled over the electrodes by capillary action. When a diabetic touches the target area, he or she has no way of knowing if sufficient blood has been transferred to run a reliable test (as discussed below, the MediSense Precision QID and LifeScan Sure Step address this problem). If not enough blood is applied to a reflectance strip, the color formation will not be complete and a low result can be given [62,63]. If not enough blood is applied to an electrochemical strip, the electrodes may not be completely covered, which would lead to a low result [62,63]. The Precision QID is the only meter that claims that more blood can be applied to the test strip if not enough is applied initially to start the assay. The diabetic can also apply an adequate amount of blood but in an improper way and obtain a low result. The reflectance meters all warn against smearing the blood onto the test strip. Smearing creates areas where thin layers of blood are applied to the strip and other areas receive normal amounts of blood. This will cause a nonuniform color to form across the reactive zone of the strip, which can lead to low results. The reflectance meters tell the diabetic to look at the appearance of the reactive area after the test is performed. Reflectance meters manuals give pictorial examples of how the reactive area should look after proper and improper applications of blood. The percentage of diabetics who bother to check the test strip after running it is not known.

Two meters were recently introduced that try to address problems related to diabetics' interaction with the test strip. The Precision QID test strip introduced by MediSense in 1995 has a means of handling the blood when it is placed on the strip that differs from that of previous electrochemical strips. The blood is deposited on a target zone on the strip and a mesh wicks the blood to the electrodes, which are not visible (see Figure 5). Having the electrodes in their own chamber means that a controlled volume of blood is delivered to the electrodes and protects the sample from evaporation. The design allows for the blood to flow over the working and dummy electrodes first. Then, only if enough blood has been added does the blood wet the reference electrode and the assay start. A study done with five meters currently on the market showed that only the Precision QID would not generate falsely low results when 1 μ L was applied to the test strip (see Figure 7).

A reflectance meter was introduced in 1996 that tries to make sure that a diabetic places the blood in the correct spot and with enough blood volume: the SureStep from LifeScan. The SureStep reverses a trend that LifeScan started: wiping the blood onto a strip and then inserting it into the meter to be read. LifeScan allowed the strip to be inserted into the meter and then the blood is applied to the reactive area on the strip. The LifeScan innovation avoided the diabetic's having to handle the strip after blood was applied except to throw it

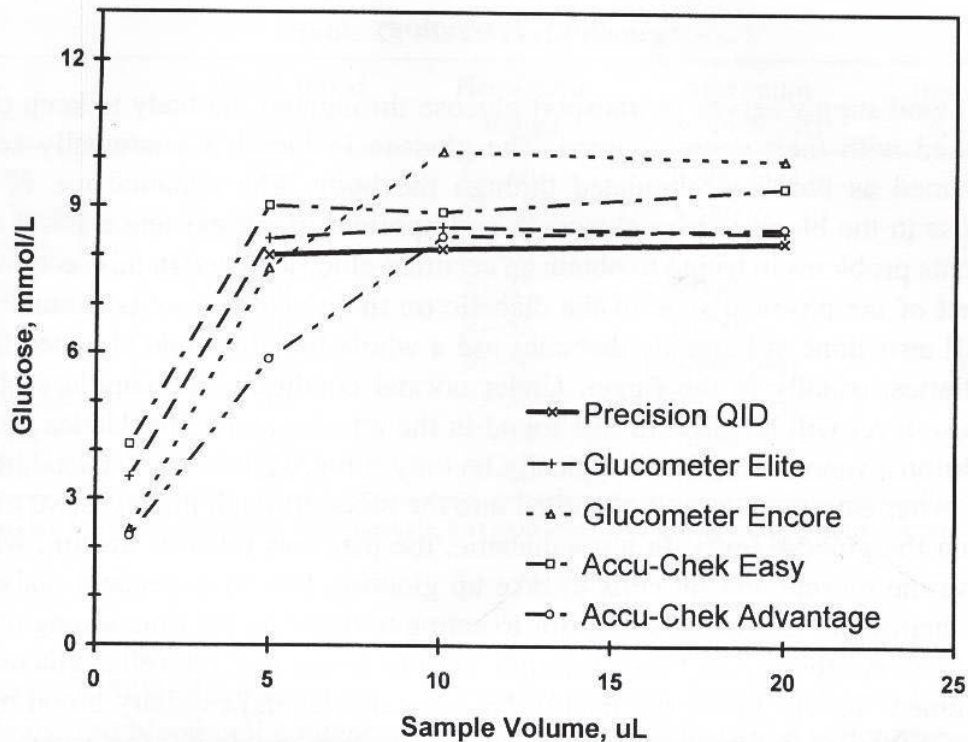


Figure 7. Effect of sample volume on glucose results. Lines connect the mean values, each calculated from 20 results, unless a test failed to start or yielded a "LO" or "Error" message. (From Ref. 63, with permission from the American Diabetes Association, Inc.)

away when the assay was finished. With the SureStep, LifeScan has returned to a strip on which the diabetic first applies blood and then inserts into the meter. The two reasons for which they did this both deal with problems the user has with application of blood to the strip. The first problem the user has is getting a blood drop onto the correct area of the strip. If the strip is inserted into the meter prior to blood application, the diabetic must try and hit the test area on the strip by manipulating the finger with the hanging drop to hit that area. The meter normally is laid flat on a table and is not moved during application of the blood. By applying the blood to the strip before insertion into the meter, the diabetic can manipulate both the finger and the test strip to apply the blood drop more easily to the reactive area on the test strip. The diabetic is also instructed to observe the color formation on the back of the strip prior to inserting the strip into the meter. If the back of the strip turns blue, the diabetic knows the blood has been applied correctly. If the strip is inserted into the meter prior to application of the blood, no visual inspection of the reactive area can be made until after the test is finished and the strip is removed for disposal. One drawback to the SureStep technology is that it is the application of too much blood is a problem.

4.6. Physiology

The blood supply serves to transport glucose throughout the body to keep cells supplied with their energy source. The glucose in blood is continually being consumed as blood is circulated through the body. The dynamic use of the glucose in the blood is why glucose is so important to our existence, but it also presents problems in trying to obtain an accurate glucose assay. In this section the impact of the physical state of the diabetic on the glucose assay is examined.

All tests done at home by diabetics use a whole-blood sample obtained from capillaries, usually in the finger. Under normal conditions the capillary blood glucose level will be close to that found in the arteries and veins. Under fasting conditions, venous blood will typically be only 5 mg/dL less than arterial blood [61]. After eating, glucose is absorbed into the blood through the digestive track, raising the glucose level. In a nondiabetic, the pancreas releases insulin, which allows the muscle and fat cells to take up glucose. Insulin-dependent diabetics give themselves an insulin shot prior to eating to replicate the functioning of the pancreas. As the blood flows through muscle tissue and fat cells, glucose is consumed. Venous blood can then differ from arterial and capillary blood by as much as 70 mg/dL after eating [61,64]. This can be a problem if the accuracy of the glucose assay run from a fingerstick is compared to blood obtained from a venous sample. Some meters also allow a venipuncture sample to be run on their meters, which is important for hospital use of the meters (see Table 7). Any medical condition that affects the circulation of the capillary system will affect the accuracy of the reading. Shock of any type (traumatic, septic, or cardiac) causes a reduction in capillary blood flow. Under conditions of shock, glucose consumption in the capillary system goes up as the flow decreases. In diabetics in shock, capillary blood samples can be much lower in glucose than arterial or venous samples may be [42,65]. Problems can also occur in diabetics suffering from dehydration [65–67]. Blood becomes thicker during dehydration and may not flow properly through the layers of the test strip for reflectance meters. This can result in low results.

One aspect of blood that has a major impact on the calibration and performance of test strips is the concentration of red blood cells. The ratio of the volume of red blood cells to the total volume is called the *hematocrit*. A normal person has a hematocrit of 43, which means that 43% of the blood volume is occupied by red blood cells. Plasma is 93% water and red blood cells are 73% water. Thus a normal blood sample with a hematocrit of 43% would be 84% water. The difference in water content between plasma and red blood cells is due primarily to the amount of volume taken up by the red cell membranes (lipid-protein cell membranes). Glucose is transported into the red cells at a rate 250 times faster than glucose is consumed inside the cell, so the glucose in plasma and red blood cells is equal. Based on the water content in plasma and blood,

Table 7. Meter Performance^a

Meter	Whole Blood Venous	Hematocrit Range	Maximum Altitude (ft)	Humidity Range
Encore	Yes	20–60	8,800 [44]	<85
One Touch	Yes	25–60	5,280	0–90
SureStep	Yes	25–65	—	10–90
Instant	No	30–55	6,000	<85
Easy	No	30–55	6,000	<85
Elite	Yes	20–60	8,800 [44]	20–80
Advantage	No	30–55	10,150	<95
ExacTech	Yes	35–55	7,200	20–80
Precision QID	Yes	30–60	7,200	10–90

^aValues are from manufacturers' user manuals, test strip inserts, or technical support unless otherwise noted.

93% versus 84%, the plasma has 11% more water and thus 11% higher glucose. Since the red blood cell content varies between individuals and is not measured by glucose meters, manufacturers must assume a normal value in their calibration.

Hematocrit is not constant and variations from 20 to 60% can be expected. The acceptable hematocrit range for each meter is listed in Table 7. Hematocrit in the range 20 to 60% would cause the plasma to read from 4.5 to 15% higher than blood [68,69]. In practice, some electrochemical strips exhibit hematocrit dependencies greater than 10% [62]. Electrochemical strips use the blood as a conductive pathway for current to flow between two electrodes. Red blood cells decrease the electrical conductivity of the blood because the red cell membranes act as insulators. This makes the electrochemical strips more prone to effects from changes in hematocrit. Changes in albumin will cause displacement of water in a manner similar to that in red blood cell membranes and have been shown to affect glucose analyzers in the clinical laboratory [70]. The effect of changes in albumin on home glucose meters has not been demonstrated [58].

4.7. Environmental Issues

Home glucose meters are portable and are designed to be carried by diabetics and used wherever they need to test their blood glucose. This again differentiates the home glucose testing market from testing done at a clinical laboratory. The glucose meters will be used under a variety of environmental conditions (temperature, humidity, altitude, etc.) that would never occur in a laboratory setting. The environmental extremes would present a challenge for even the most sophis-

ticated instrumentation but present an even bigger challenge for the inexpensive meters that are required for home glucose testing.

Altitude has been identified as a problem for some meters [71]. Most meters reference a known altitude at which they can be used without accuracy problems (see Table 7). At higher altitudes the oxygen content of air is lower. Test strips that use oxygen as a cofactor for glucose oxidase may be affected by altitude. The color formation reaction of some reflectance strips may not reach an endpoint, due to less oxygen being available. Test strips that use a mediator instead of oxygen as a cofactor for glucose oxidase or use glucose dehydrogenase show very little effects from altitude [44]. Humidity is another environmental factor that can affect performance. Bringing a cold meter or strips into a warm house can cause condensation on the meter and strips. Condensation on strips is a problem because the condensate will be analyzed with the blood. Condensation on the optics of a reflectance meter is also a problem. All meters list a maximum humidity specification (see Table 7), which is typically between 85 and 90%. Humidity also affects the stability of the test strips. All manufacturers use techniques designed to reduce the humidity in the container in which the test strips are stored. Test strips that come packaged in a vial have a desiccant in the cap of the vial to maintain low humidity during storage. Manufacturers warn against leaving the cap off for any length of time. Other manufacturers have chosen to package their strips in individual foil packages, some containing their own desiccant for each strip.

Each glucose test strip works by a chemical reaction involving glucose, an enzyme, and at least one additional reaction designed to produce a measurable signal. All the chemical reactions are temperature dependent. There is no cheap way of monitoring the reaction temperature occurring on a test strip. Manufacturers include sensors in their instrument which can monitor the ambient temperature and make corrections based on the ambient temperature. One manufacturer has patented algorithms for temperature corrections [72]. The BMC Advantage and Instant will flash a thermometer on their display if the temperature is outside the operation conditions for the glucose test, 64 to 90°F. This is used to indicate that the test result may be invalid due to the ambient temperature.

4.8. Performance Summary

Home glucose meters do not achieve the 15% accuracy that the American Diabetes Association would like. The Consensus Statement on Self-Monitoring of Blood Glucose states [39]: "Approximately 50–70% of individuals who receive some sort of formal training are capable of obtaining a result within 20% of the reference method: however performance may deteriorate over time." This section on performance has explained why a glucose biosensor being used by average people during their daily life can create problems for accuracy and precision. The

challenge exists for glucose meter manufacturers to improve performance. The fact should not be lost that millions of diabetics are using these meters today to improve the quality of their life and at times to save a life. The glucose biosensor remains the most financially successful and most successful in improving the human condition. Present problems with glucose biosensors represent opportunities for future generations of biosensors.

5. FUTURE DIRECTIONS

There are a great number of publications concerning the determination of glucose. Of possible importance is work to develop other biochemistries and electron transfer systems. Concanavalin A selectively binds to glucose and may be conjugated to easily detectable reagents [16]. Glucose dehydrogenase has been isolated with an activity 20-fold greater than that of pure glucose oxidase [73,74]. Interestingly, many of the mediators useful with the glucose oxidase-FAD system also function in the glucose dehydrogenase-PQQ system [75]. Many other mediator systems have been reported for glucose oxidase. Some organometallic compounds are efficient mediators and have been incorporated into polymer matrices for the construction of reusable sensors. Synthetic metals and electrically conducting organic polymers have been identified as possibly being useful for direct electron transfer to the redox active center of the enzyme [76-79].

Significant efforts have been made to develop implantable sensors [80-82]. The ultimate goal is to develop biofeedback systems where the sensor controls an insulin pump or an implanted biohybrid artificial pancreas [83,84]. A less invasive measurement system using reverse iontophoresis technology is currently under development [85]. Other efforts have focused on development of noninvasive methods of analysis. Near-infrared and infrared systems have been developed, but have not been commercialized to date. Low-power radio-frequency-wave and other technologies are currently being tested. It is not clear which technologies will enter the marketplace. Certainly, the development of biosensors for personal diabetes management will remain an active, challenging, exciting, and rewarding field.

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