

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HELENA LABORATORIES CORPORATION

Petitioner,

v.

SEBIA

Patentee.

Case No.:

PETITION FOR *INTER PARTES* REVIEW OF

U.S. PATENT NO. 7,887,686

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List of Evidence and Exhibits Relied Upon in the Petition

Exhibit #	Name
1001	U.S. Patent No. 7,887,686 (the “‘686 Patent”). to Robert <i>et al</i>
1002	Power of Attorney for Petitioner
1003	File History of U.S. Patent Application No. 11/280,025, filed November 16, 2005.
1004	Declaration of Alan Wu, Ph.D.
1005	<i>Curriculum Vitae</i> of Alan Wu, Ph.D.
1006	Noriaki Ishioka <i>et al</i> , “Detection of Abnormal Haemoglobin by Capillary Electrophoresis and Structural Identification.” <i>Biomedical Chromatography</i> (1992). Vol. 6, pages 224-226.
1007	Ahmet Sahin <i>et al</i> , “Haemoglobin analysis by capillary zone electrophoresis.” <i>Journal of Chromatography</i> (1995). pages 121-125.
1008	Margaret A. Jenkins <i>et al</i> , “Capillary isoelectric focusing of haemoglobin variants in the clinical laboratory.” <i>Clinica Chimica Acta</i> (1999). Vol. 289, pages 121-132.
1009	Margaret A. Jenkins <i>et al</i> , “Evaluation of hemoglobin A ₂ quantitation assay and hemoglobin variant screening by capillary electrophoresis.” <i>Journal of Capillary Electrophoresis</i> (1997). 004:3, pages 137-143.
1010	Margaret A. Jenkins <i>et al</i> , “Review: Capillary electrophoresis as a clinical tool.” <i>Journal of Chromatography</i> (1996). pages 23-34
1011	Zak K. Shihabi <i>et al</i> , “Hemoglobin A ₂ quantification by capillary zone electrophoresis” <i>Electrophoresis</i> (2000). Vol. 21, pages 749-752.
1012	James M. Hempe <i>et al</i> , “Separation of hemoglobin variants with similar charge by capillary isoelectric focusing: Value of isoelectric point for identification of common and uncommon hemoglobin variants.” <i>Electrophoresis</i> (2000)., Vol. 21, pages 746-748.

1013	Coufal <i>et al</i> , “The Magnitude and Reproducibility of the Electroosmotic Flow in Silica Capillary Tubes” <i>Journal of High Resolution Chromatography</i> , May 1994, Vol. 17, pages 325-334.
1014	Fang <i>et al</i> “Use of Zwitterionic Buffer Additives to Improve the Separation of Proteins in Capillary Zone Electrophoresis” <i>Journal of High Resolution Chromatography</i> , November 1994, Vol. 17, pages 749-752.
1015	Huang <i>et al</i> “The Separation of Hemoglobin Variants by Capillary Zone Electrophoresis” <i>Journal of High Resolution Chromatography</i> , Vol. 17, pages 675 – 678.
1016	U.S. Patent No. 5,202,006, Chen
1017	U.S. Patent No 5,439,825, Chen
1018	U.S. Patent No. 5,536,382, Sunzeri
1019	Mohammad <i>et al</i> “Clinical Application of Capillary Isoelectric Focusing on Fused Silica Capillary for Determination of Hemoglobin Variants” <i>Clinical Chemistry</i> , 1997, Vol. 43, pages 1798-1799`
1020	Camilleri “ <i>Capillary Electrophoresis</i> ” 1997, pp 370-371
1021	U. S. Patent No. 6,08,3782, Grover
1022	U.S. Patent No. 5,753,095, Alter
1023	U.S. Patent Publication 2002/016274, Nouadje
1024	U.S. Patent No. 7,297,244, Nouadje

1025	Song <i>et al</i> "Improved capillary zone electrophoretic separation of basic proteins in uncoated fused-silica capillary by using ethylene diamine as a buffer additive" <i>Journal of Chromatography</i> , 1993, Pages 175-183
1026	Landers <i>et al</i> "High-Performance Capillary Electrophoresis of Glycoproteins: The Use of Modifiers of Electroosmotic Flow for Analysis of Microheterogeneity" <i>Analytical Chemistry</i> 1992, Vol 205, Pages 115-124

I. ACRONYMS

CE	Capillary Electrophoresis
CZE	Capillary Zone Electrophoresis
DAB ¹	1,4-diaminobutane
DAP	1,5-diaminopentane
EOF	Electroosmotic Flow
FI	Flow Inhibitor
Hb	Hemoglobin
POSITA	Person of ordinary skill in the art
ZB	Zwitterionic Buffer

II. INTRODUCTION

U.S. Patent No. 7,887,686 (the “’686 Patent”). (Ex.1001). relates to separating hemoglobin A₂ (HbA₂). in a blood sample from other hemoglobin variants in the blood sample (*e.g.*, HbC, HbE, HbS and HbD)., by capillary electrophoresis (CE)., and then quantifying the amount of the hemoglobin A₂.

¹ The following “color coding” is used in this Petition and/or in the accompanying Exhibits for correlating claim language (and/or relevant functions) to the prior art. Not all occurrences are color coded. The color coded terms are ZB (including examples of ZB), FI (including examples of FI), Hb, pH (including modification or adjustment of pH), EOF (or flow), and concentration (molarity).

Electrophoresis is the movement (migration). of particles under the influence of an electric field. CE is electrophoresis through an elongated hollow tube referred to as a capillary. The tube is filled with a buffer solution and the sample is placed in the buffer solution. The sample flows through the tube under the influence of the electric field. The movement of the sample within the tube is referred to as electroosmotic flow. (EOF).

The terms CE and CZE are used interchangeably. The '686 patent describes the limitations of prior art CE techniques for separating hemoglobin A₂ from other variants in terms of low resolution (*i.e.*, insufficient separation). based on several prior art documents.

The '686 patent asserts that it solves those problems by using a buffer having both zwitterionic and FI components, *i.e.*, ZB and FI. The FI reduces the EOF through the CE tube thus allowing more time for better separation among the variants.

There are no drawings of a CE system in the '686 patent nor were any required. CE was a well-known technique, and the patent explains the use of conventional CE equipment.

Each claim in the '686 patent is directed to the method of hemoglobin separation by CE. Two steps are required: First, the sample is introduced into the CE tube where there is combination of a ZB and an FI. Second, electrophoresis, *i.e.*

separation of the sample components, is performed by applying voltage to subject the sample to the electric field.

Missing from prior art applied to the claims during the pendency of the application are:

- a) Hemoglobin variant separation by CE alone or with ZB;
- b) Any CE separation using both ZB and FI.
- c) Any CE separation recognizing the need to reduce EOF.
- d) Any CE separation using ZB and FI to reduce EOF.

This Petition provides the evidence for each of the above.

Shihabi (Ex. 1011). teaches CE Hb variant separation with a two component buffer, one component being a ZB and the second not being an FI.²

Huang (Ex. 1015). teaches CE Hb variant separation using ZB and teaches the need to reduce EOF (accomplished by modifying pH and voltage) to enhance separation of the variants.

Coufal (Ex. 1013). teaches a CE analysis buffer combination of ZB and FI to reduce EOF for improved separation of the components of the sample.

Camilleri (Ex. 1020). provides a “laundry list” of potential ZB and potential

² Shihabi was mentioned in the ‘686 patent but was not applied by the Examiner.

FI used in CE.

The challenged claims would have been obvious to a POSITA, at the time of the invention, over either of two primary references Shihabi, or Huang when combined with the teachings of Coufal (and with additional references for various dependent claims).

Specifically, various dependent claims address features such as **pH range**, **modification of pH**, specific **ZBs**, **concentrations** of **ZB**, specific **FIs**, concentrations of **FI**, etc. Some of those dependent claim features are not found in Shihabi, Huang or Coufal but were part of the prior art as confirmed by the other references such as Camilleri (Ex. 1020) and Landers (Ex. 1026).

This Petition will show that there is a reasonable likelihood that all claims of the '686 Patent are unpatentable as follows (Claims 1, 23 and 24 are independent):

1. Claims 1, 2, 3, 6, 7, 10 – 14, 17, 18 and 23 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in view of Coufal (Ex. 1013)(Ex. 1004 ¶101, pages 1004-0045 through 1004-0046);

2. Claims 15 and 16 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in

view of Coufal (Ex. 1013) and Camilleri (Ex. 1020)(Ex. 1004 ¶119, page 1004-0066 through 1004-0067); and

3. Claims 4, 5, 8, 9, 19-22 and 24 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in view of Coufal (Ex. 1013) and Landers (Ex. 1026) (Ex. 1004 ¶124 page 1004-0068 through 1004-0069).

III. MANDATORY NOTICES AND FEES

The following mandatory notices, power of attorney and fee payment information required under Rules 42.8, 42.10, and 42.15 are provided.

A. *Real Party-in-Interest Under 37 C.F.R. §42.8(b)(1).*

Petitioner certifies and submits that the real party-in-interest for this Petition is Petitioner Helena Laboratories Corporation, a Texas (U.S.) corporation.

B. *Related Matters Under 37 C.F.R. §42.8(b)(2).*

There are no related matters that would affect or be affected by a decision in the present proceeding.

C. *Lead and Back-Up Counsel Under 37 C.F.R. §42.8(b)(3).*

Petitioner designates lead and backup counsel as:

<u>Lead Counsel</u>	<u>Backup Counsel</u>
Jerold I. Schneider Reg. No. 24,765 Schneider IP Law 21048 95 th Ave S. Suite D-206 Boca Raton, FL 33428-1567 Phone: 561-309-5374 jerold.schneider@schneideriplaw.legal	Scott D. Smiley Reg. No. 55,627 The Concept Law Group, P.A. 6400 North Andrews Avenue Suite 500 Fort Lauderdale, FL 33309 Phone: 754-300-1500 scott@conceptlaw.com

D. *Service Information Under 37 C.F.R. §42.8(b)(4).*

Papers concerning this matter should be served on lead and backup counsel as designated in Section II(C). Petitioner hereby consents to email service of any papers in this proceeding.

E. *Power of Attorney Under 37 C.F.R. §42.10(b).*

A Power of Attorney executed on behalf of Petitioner is filed concurrently with this Petition in accordance with 37 CFR §42.10(b) (Ex.1002).

F. *Payment of Fees Under 37 C.F.R. §42.15(a).*

This Petition requests review of all 24 claims of the ‘686 Patent. A payment

of \$46,000 is made concurrently via the USPTO EFS payment system, which comprises a \$20,500 request fee (for 24 claims)., and a post-institution fee of \$25,500 (for 24 claims), for a total of \$46,000. This Petition meets the fee requirements of 35 U.S.C. §312 (a)(1).

IV. SUMMARY OF THE ‘686 PATENT

A. Description of the Claimed Invention of the ‘686 Patent

Independent claim 1 of the ‘686 patent conceptually has two steps, the first being “introducing a sample... into a capillary tube ... comprising a solution of an analysis buffer” and the second being “electrophoresing the sample in said analysis buffer...” - - and the buffer in each step includes the ZB and FI. (Ex. 1001-0019).³

Independent claim 23 has the same two steps but the preamble of claim 23 provides details of the sample – details which also appear in dependent claim 13. (Id.).

Independent claim 24 has the same two steps but requires the FI to be of the

³ Pursuant to 37 C.F.R. §42.63(c) and (d), each Exhibit is uniquely numbered, and each page is uniquely numbered. The unique numbering comprises the four digit Exhibit number as a “prefix” followed by a four digit pagination. Thus page 19 of Exhibit 1001 is uniquely numbered as 1001-0019.

aliphatic or cyclic diamine or polyamine type – details which also appear in dependent claims 4 and 5. (Id.).

The ‘686 patent provides examples of “known, commercially available” ZB, referring to them as “Tris” type (Ex. 1001-0018, Column 3, line 54 – Column 4, line 7). Significantly, the Tris type ZB was used in two prior art systems described in the ‘686 patent: a Tris buffer in the Sahin system (Ex. 1007-0002) and a Tris/arginine buffer combination in the Shihabi system. (Ex. 1011-0001).

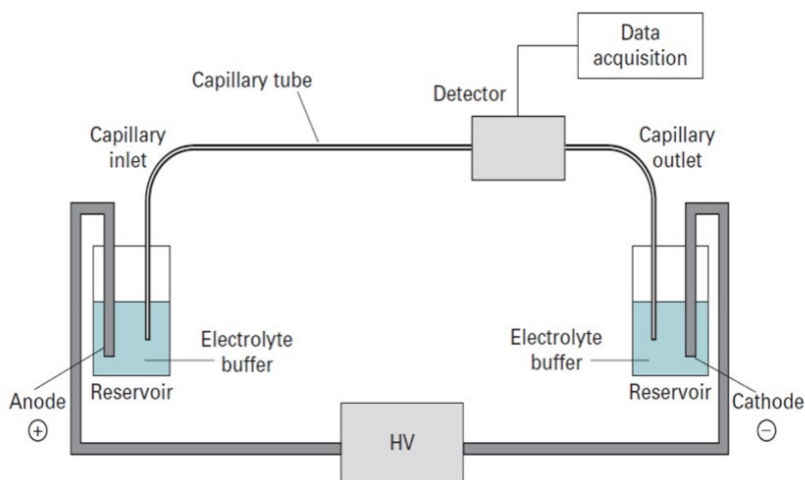
The ‘686 patent provides examples of FI including DAB and DAP. (Ex. 1001-0017 and 1001-0018, Col. 2, lines 40 – 58 and Col. 4, lines 8 – 14) both of which were used in prior art capillary electrophoresis systems (Ex. 1020, Ex.1026).

B. *An overview of capillary electrophoresis*

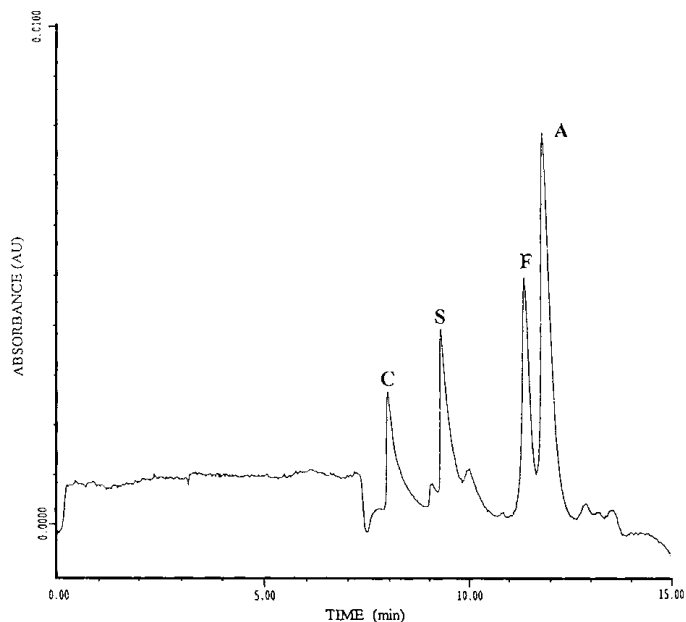
Capillary electrophoresis is a well-known procedure taught as part of analytical chemistry to undergraduate chemistry students. (Ex. 1004, ¶¶18, 32; 37 pages 1004-0010, 1004-0016, 1004-0018).

The following is an illustration of a CE system. The sample to be evaluated is placed in a buffer solution (sometimes referred to as an electrolyte) and a voltage is applied between the anode (+). and the cathode (-). The voltage causes the sample to flow through the buffer within the capillary tube (*i.e.*, migrate) from the anode to the cathode. This is referred to as electroosmotic flow or EOF. Ions in the

sample are separated by their electrophoretic mobility which, in turn, is a function of the charge of the molecule, the viscosity, and the radius of the atom. As the separated components or constituents of the sample flow through the tube, they are illuminated and detected to obtain qualitative and quantitative information. The sample then proceeds to the outlet of the tube. The region where the components are illuminated is referred to as the CE “zone” thus the terms CE and CZE are used interchangeably. (Ex. 1004 ¶33, page 1004-0017).



The output or result of capillary electrophoresis of hemoglobin is illustrated in in following from Ex. 1015 using standard CE equipment with the four variants HbA, HbF, HbS and HbC identified. (Ex. 1004-¶¶35-36, page 1004-0018).



C. Summary of the Prosecution History of the ‘686 Patent

The entire prosecution history of the ‘686 Patent is of record as Ex.1003. U.S. Patent Appl. No. 11/280,025 was filed on November 16, 2005, claiming priority to French Patent Application No. 04 12263 filed on November 18, 2004. For the purpose of this Petition, November 18, 2004, will be considered the “invention date” unless and until an earlier date is established by the inventors/assignee.

The ‘686 patent contained 28 original claims.(Ex.1003-0017 *et seq.*; Ex. 1004 ¶43, page 1004-0027). The claims were replaced with claims 29 – 55 (Ex. 1003-0203 *et seq* Ex. 1004 ¶43, page 1004-1027) prior to any evaluation by the

Examiner. The proceedings were limited to claims 29-52 (Ex. 1003-0240 *et seq* Ex. 1004 ¶43, page 1004-0027).

Claims 29-52 were rejected multiple times based on a patent publication to Nouadje *et al* (U.S. 2002/0162744) relating to the use of a ZB combined with Grover (U.S. Patent 6,083,372) relating to DAB as the FI. Applicant argued, ultimately successfully, that Nouadje contained a translation error and actually related to serum or plasma but was not for separating hemoglobin (blood) proteins. (Ex. 1003-0372 *et seq*; 1003-04011 Ex. 1004 ¶44, page 1004-0027).

All prior rejections were withdrawn and there was a new rejection based on Sunzeri (U.S. Patent 5,536,382 (Ex. 1018)(Ex.1003-0412 *et seq*). as prior art for CE of hemoglobin A1c separation and detection with a ZB (tricine) and a quaternary amine for flow reversal. (Emphasis added). The Examiner indicated that Camilleri publication (Capillary Electrophoresis, 1997, Ex. 1020-0370 to 1020-0371) taught the combination of a ZB (tricine) in a concentration of up to 1 M, and DAB as a FI with a concentration up to 60mM. (Ex. 1003-0415- Ex.1003-0418 Ex.1004 ¶46, page 1004-0028).

The response successfully argued that Sunzeri detects an analyte after the analyte has been bound with a labeled reagent (*i.e.*, resolving the difference between uncomplexed antibody from an antibody-antigen complex). In other words, Sunzeri detects a single, specific hemoglobin variant rather than separating

and resolving multiple hemoglobin variants. The response further argued that the Camilleri publication functioned to reduce protein absorption on the capillary surface and described increasing the concentration of the quaternary amine CTAB to reverse the charge and thus reverse the flow and distinguished that from the claimed **FI** that reduces EOF. (Id., ¶ 47, page 1004-0028). As a result of these arguments, the '686 Patent was granted.

V. REQUIREMENTS UNDER 37 C.F.R. §42.104

Petitioner submits that each requirement for institution of an *inter partes* review (IPR). of the '686 Patent is satisfied.

A. *Grounds for Standing Pursuant to 37 C.F.R. §42.104(a).*

Petitioner certifies that the '686 Patent is available for IPR, and that Petitioner, which is the only real-party-in-interest, is not barred or estopped from filing this Petition (Ex.1002).

B. *Identification of Challenged Claims and Relief Requested Pursuant to 37 C.F.R. §42.104(b).(1).*

The precise relief requested by Petitioner is that claims 1- 24 of the '686 Patent (the challenged claims) be found unpatentable.

C. *The Specific Art and Statutory Grounds on Which the Challenge is Based Pursuant to 37 C.F.R. §42.104(b).(2).*

Petitioner requests an IPR of the '686 Patent be instituted on the grounds of unpatentability under 35 U.S.C. §103 (obviousness):

Ground 1: Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in view of Coufal (Ex. 1013). (Ex. 1004 ¶101, page 1004-0045).

Ground 2: Claims 15 and 16 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in view of Coufal (Ex. 1013) and Camilleri (Ex. 1020). (Ex. 1004 ¶ 119, page 1004-0066-67).

Ground 3: Claims 4, 5, 8, 9, 19-22 and 24 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in view of Coufal (Ex. 1013) and Landers (Ex. 1026). (Ex. 1004 ¶ 124, page 1004-0068-69).

D. *How the Challenged Claims are Construed Pursuant to 37 C.F.R. §42.104(b).(3).*

A claim in an IPR is construed using the same claim construction standard

that would be used to construe the claim in a civil action, including construing the claim in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent. 37 C.F.R. §42.100.

One term in the patent may need construction. The term is “flow inhibitor”. Sebia explained “flow inhibitor” in response to a rejection as follows:

“The flow inhibitor is not specific for a particular analyte or a complex to be separated, but interacts with the capillary, thus *reducing* the electroosmotic flow (EOF). and displacing each compound present in the capillary in a homothetic manner, *i.e.*, the variants remain in the same order when separated.” (Emphasis in original).

Sebia continued “the present invention comprises the use of an analysis buffer of the zwitterionic type which comprises at least one flow inhibitor which is used to *decrease* the charge density on the capillary surface and thus *reduce* the EOF.” (Emphasis in original).(Ex. 1002, page 0461).

Thus, Petitioner submits that the proper construction of “flow inhibitor” is a

substance which is not specific to the components being separated and which **reduces or decreases** the EOF. (Ex. 1004 ¶¶80-81, page 1004-0038).

The fact that the **FI** is not specific to the substance being separated is also important in defining the relevant art. The relevant art is not limited to hemoglobin separation by CE, rather, the relevant art is CE *per se*. (Ex. 1004 ¶82, page 1004-0038).

VI. DETAILED EXPLANATION OF PERTINENCE AND MANNER OF APPLYING CITED PRIOR ART TO EVERY CLAIM FOR WHICH REVIEW IS REQUESTED

A. Overview of the Prior Art

The claims relate to a CE method of determining hemoglobin variants with a **ZB** and an **FI**. The dependent claims add features such as the **pH** of the buffer solution, **adjusting (modifying). the pH, concentration** of the **ZB, concentration** of the **FI**, specific **ZB** chemicals, and specific **FI** chemicals.

The '686 Patent identifies the discovery as the addition of **FI** to the **ZB** for CE separation of hemoglobin variants. (Ex. 1001-0017, Col. 2, lines 11-16). (Ex. 1004 ¶69, page 1004-0034).

Without limiting Petitioner's right to rely on any exhibit filed with this Petition for any purpose, Petitioner points out the following:

1. **Shihabi (Ex. 1011).** Zak K. Shihabi *et al*, “Hemoglobin A₂ quantification by capillary zone electrophoresis” *Electrophoresis* (2000). Vol. 21, pages 749-752. Shihabi teaches CE separation of hemoglobin variants using ZB and a buffer additive (not an FI), a pH of 8.5 (within the range of a narrower dependent claim of the ‘686 patent); modifying or adjusting the pH (referred to in a dependent claim of the ‘686 patent); Tris as the ZB (referred to in a narrower dependent claim of the ‘686 patent), and that better separation of the hemoglobin variants occurs with the buffer additive. (Ex. 1004 ¶95, page 1004-0043).

This prior art document was mentioned in the background of the ‘686 patent but never applied against any of the claims. (Id.).

2. **Huang (Ex. 1015).** Tung-Liang Huang *et al* “The Separation of Hemoglobin Variants by Capillary Zone Electrophoresis” *Journal of High Resolution Chromatography*, Vol. 17, pages 675 – 678. Huang teaches CE separation of hemoglobin variants using ZB, modifying or adjusting the pH (referred to in a dependent claim of the ‘686 patent); Tris and HEPES as alternative ZB (referred to in narrower dependent claims of the ‘686 patent), and that better separation of the hemoglobin variants occurs with the buffer additive. (Ex. 1004 ¶96, page 1004-0043-44).

This prior art document was not cited by Sebia or the Examiner and is newly

cited prior art. (Id.).

3. Coufal (Ex. 1013). Pavel Coufal *et al*, “The Magnitude and Reproducibility of the Electroosmotic Flow in Silica Capillary Tubes” *Journal of High Resolution Chromatography*, May 1994, Vol. 17, pages 325-334. Coufal teaches basic principles of CE separation, the importance of buffer selection, the use of ZB and a FI, a pH within the range of the narrower dependent claim of the ‘686 patent); modifying or adjusting the pH (referred to in a dependent claim of the ‘686 patent); Tris as a preferred ZB (referred to in narrower dependent claims of the ‘686 patent); a concentration of ZB within the range of a narrower dependent claim of the ‘686 patent; two different FI, one of which falls within the narrower dependent claims of the ‘686 patent, and that better CE separation occurs with the use of a buffer additive. (Ex. 1004 ¶97, page 1004-0044).

This prior art document was not cited by Sebia or the Examiner and is newly cited prior art. (Id.).

4. Camilleri (Ex. 1020). Camilleri “*Capillary Electrophoresis*” 1997, pp 370-371. This textbook teaches various choices of ZB (including Tricine which is in a narrower dependent claim of the ‘686 patent) and various choices of FI including DAB (which is in a narrower dependent claim of the ‘686 patent) for reducing EOF. (Ex. 1004 ¶91, page 1004-0041).

This prior art document was cited by the Examiner. Sebia successfully

argued only that a different portion of Camilleri taught the use of CTAB for reversing the EOF.(Ex. 1004, ¶47, page 1004-0028-29).

5. Landers (Ex. 2026). Landers *et al* “High-Performance Capillary Electrophoresis of Glycoproteins: The Use of Modifiers of Electroosmotic Flow for Analysis of Microheterogeneity” *Analytical Chemistry* 1992, Vol 205, Pages 115-124. Landers teaches the use of **DAB** as a **FI** within the narrower scope of a dependent claim in the ‘686 patent and at an **FI concentration** of 0.250mM (also within the scope of a narrower dependent claim). (Ex. 1004 ¶73, page 1004-0036). This prior art document was not cited by Sebia or the Examiner.

The prior art illustrates that the purportedly novel and non-obvious feature, the addition of an **FI** in the CE buffer to reduce EOF had been well-studied and well-published and was well-known and obvious to a POSITA long prior to the invention date of the ‘686 patent.

B. *Person of Ordinary Skill in the Art.*

A POSITA at the relevant time frame, would (1) have a baccalaureate degree in Chemistry, (2) be actively seeking (or have already received). an advanced related scientific degree, and (3) have at least two years of experience in the capillary electrophoresis, or many years of experience in the art and less formal education. (Ex. 1004 ¶¶63-64, page 1004-0032-33). The POSITA would have studied separation techniques including electrophoresis (both gel and capillary) and

would be familiar with CE equipment and its use and limitations, and would be experienced in applying scientific approaches, principles and techniques to problem solving. (Id.).

C. *The relevant art.*

The relevant art is analytical chemistry, *i.e.*, determining the chemical composition and structure of materials and the quantitative and qualitative evaluation of the amounts of those materials and, for example, separation by CE, but not limited to separation of hemoglobin variants by CE. (Ex. 1004 ¶¶61, page 1004-0032 and ¶¶82, page 1004-0038).

VII. Claim-by-claim Obviousness Analysis.

A. GROUND 1: Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015), each in view of Coufal (Ex. 1013). (Ex. 1004 ¶¶101, page 1004-0044-45).

Shihabi and Huang each teach all of the features of claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23 and the need for reducing EOF to achieve better separation of the components of the sample, but do not teach a FI buffer additive. Coufal teaches various ZB/FI combinations and FI buffer additives to reduce EOF and thus increase the separation of the components of the sample. (Ex. 1004 ¶¶102,

page 1004-0046).

It would have been obvious to a POSITA to modify Shihabi or Huang by adding the **FI** of Coufal. (Ex. 1004 ¶ 103, page 1004-0046). The obviousness analysis under *Graham* first requires a determination of the scope and content of the prior art. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). The scope of the prior art includes art that is reasonably pertinent to the particular problem with which the invention was involved. Shihabi and Huang both relate to CE separation of hemoglobin variants and the need for modification of EOF for improved separation of the components and Coufal teaches basic principles of CE separation. It should be remembered that **FI** has been explained by Sebia as being independent of the nature of the sample being separated. (See V.D. above). (Ex. 1004 ¶103, page 1004-0046).

Shihabi and Huang each teach a hemoglobin variant separation by CE, identical to the claims of the '686 Patent except for the FI to enhance separation, a feature that is expressly taught by Coufal for CE. (Id.).

Because adding the **FI** yields the predictable results of reducing EOF and thus increasing separation of the components of the sample, the claims merely set forth old steps and old components, with each performing the same function it had been known to perform and yielding no more than one would expect from such an arrangement. Thus, the claims of the '686 Patent enumerated in this GROUND 1

would have been obvious to a POSITA prior to the invention date. (Ex. 1004 ¶104, page 1004-0046-47).

Accordingly, the claims in this GROUND 1 would have been obvious under 35 U.S.C. §103 to a POSITA prior to the invention date as explained in the following claim charts.

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
A free solution CE method for separating and resolving haemoglobins in a biological sample by CE, said biological sample being a blood sample, said method comprising:	Shihabi teaches everything in the preamble of the claim. (Ex. 1004 ¶105 page 1004-0047).	Huang teaches everything in the preamble of the claim. (Ex. 1004 ¶105 page 1004-0047).
introducing said biological sample into a capillary tube comprising a solution	Shihabi teaches everything in the first step of the method including use of an arginine-Tris buffer (Tris is	Huang teaches everything in the first step of the method including the use of a ZB (bis-tris propane)

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
of an analysis buffer, wherein said analysis buffer is of the zwitterionic type and at least one FI, and	a ZB), but Shihabi does not teach the use of the FI. (Ex. 1004 ¶105, page 1004-0047). Coufal teaches use, in capillary columns, of a solution including a ZB (Tris) and including at least one flow inhibitor (ethylenediamine) and that another FI (SDS) provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013-0001 (Summary). and 1013-0009). (Ex. 1004 ¶105, page 1004-0048).	and that “reducing both the EOF and the electrophoretic mobilities of the two species can maximize the resolution (at the expense of longer separation time” (Ex. 1015-0001 0001) but does teach the use of the FI. (Ex. 1004 ¶105, page 1004-.0047). Coufal teaches use, in capillary columns, of a solution including a ZB buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
		another FI (SDS). provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013-0001 (Summary) and 1013-0009); (Ex. 1004 ¶105, page 1004-0048).
electrophoresing said biological sample in said analysis buffer and at least one said FI whereby said haemoglobins are separated by migration, and wherein said haemoglobins are a haemoglobin HbA2 and an HbC, an HbD,	Shihabi identifies the separated hemoglobin variants A, F, S, and C and A₂ (Ex. 1011-0001) and that separation was better with a Tris buffer having an additive (arginine) as compared to a plain Tris buffer. (Ex. 1001-0002). (Ex. 1004 ¶105, page 1004-0049).	Huang teaches the step of electrophoresing and that the hemoglobin variants are A, F, S and C (Ex. 1015-0002, Figure 1 and Table 1); (Ex. 1004 ¶105, page 1004-0049).

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
an HbE, an HbS, an HbF or an HbA haemoglobin.		

To a POSITA, the invention of claim 1 would have been obvious as of the invention date because it only uses familiar **ZB** and **FI** buffer components previously used in CE, and with no change in their respective functions that they had been known to perform and providing the predictable result of reducing EOF thus providing better separation of the components of the sample when used in hemoglobin CE.

Claim 2	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, further comprising	See the above chart for this GROUND 1 for claim 1. (Ex. 1004 ¶106, page 1004-0050).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004 ¶106, page 1004-0050).
detecting said haemoglobins.	Shihabi detects the separated hemoglobin variants A, F, S, and C and	Huang detects the hemoglobins A, F, S and C (Ex. 1015-0002, Figure 1

Claim 2	Shihabi in view of Coufal	Huang in view of Coufal
	A ₂ (Ex 1011-0002, Fig 1; Ex. 1004 ¶106, page 1004-0050).	and Table 1; Ex. 1004 ¶106, page 1004-0050).).

To a POSITA, the invention of claim 2 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it only uses familiar **ZB** and **FI** buffer components previously used in CE, and with no change in their respective functions that they had been known to perform and providing the predictable result of reducing EOF thus providing better separation and thus better detection of the components of the sample when used in hemoglobin CE.

Claim 3	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1.(Ex. 1004; ¶107, page 1004-0051).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004; ¶107, page 1004-0051).
the ZB is formed by one or two molecules	Shihabi teaches use of a Tris buffer . (Ex.1011-	Huang teaches use of a bis-tris propane ZB . (Ex. 1015

Claim 3	Shihabi in view of Coufal	Huang in view of Coufal
and comprises at least one amine function, at least one acid function and at least one hydroxyl function in the position opposite to the acid function.	0001; Ex. 1004; ¶107, page 1004-0051). Coufal teaches use of a tris buffer. (Ex. 1013-0001, Ex. 1004, <i>Id.</i>). The ‘686 patent confirms that a bis-tris propane zwitterionic analysis buffer is formed by one or two molecules and comprises at least one amine function, at least one acid function and at least one hydroxyl function in the position opposite to the acid function. (Ex. 1001-0017, Col. 3, lines 54-64; Ex. 1004 ¶ 107, page 1004-0051).	-0001; Ex. 1004; ¶107, page 1004-0051). Coufal teaches use of a tris buffer. (Ex. 1013-0001, Ex. 1004, <i>Id.</i>). The ‘686 patent explains that a bis-tris propane zwitterionic analysis buffer is formed by one or two molecules and comprises at least one amine function, at least one acid function and at least one hydroxyl function in the position opposite to the acid function. (Ex. 1001-0017, Col. 3, lines 54-64; Ex. 1004, page 1004-0051-52).

To a POSITA, the invention of claim 3 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it only uses a prior art ZB previously used in CE, and with no change in the function that the ZB had been known to perform and no change in the result.

Claim 6	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1. (Ex. 1004 ¶ 108, page 1004-0052).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004, ¶ 108, page 1004-0052).
said analysis buffer has a concentration in said analysis buffer solution from about 20 mM to about 500 mM.	The ZB concentration in Coufal is 20mM (Ex. 1013, Table 1). CE buffers (non-ZB). within this range (e.g., 100 mM) are confirmed as prior art by the '686 patent. (Ex. 1001-0016; Ex. 1004, ¶ 108, page 1004-0052). The range in the claim	The ZB concentration in Coufal is 20mM (Ex. 1013, Table 1). CE buffers (non-ZB). within this range (e.g., 100mM) are confirmed as prior art by the '686 patent. (Ex. 1001-0016; Ex. 1004, ¶ 108 page 1004-0052). The range in the claim

	would have been obvious to a POSITA as there does not appear to be any criticality of the range and/or its end points. (Ex. 1004 ¶ 108, page 1004-0053). The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).	would have been obvious to a POSITA as there does not appear to be any criticality of the range and/or its end points. (Ex. 1004 ¶ 108, page 1004-0053). The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).
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To a POSITA, the invention of claim 6 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it only uses a prior art **ZB** previously used in CE, and at a **concentration** previously used in CE, with no change in the prior art function that it had been known to perform and no change in the prior art result attributable to the **ZB**.

Claim 7	Shihabi in view of Coufal	Huang in view of Coufal
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The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1. (Ex. 1004 ¶ 109, page 1004-0052).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004 ¶ 109, page 1004-0052).
said analysis buffer has a concentration in said analysis buffer solution from about 100 mM to about 250 mM.	Buffers (non-ZB). within this range are confirmed as prior art by the '686 patent. (Ex.1001-0016; Ex. 1004 ¶ 109, page 1004-0053); (Ex. 1004 ¶ 109, page 1004-0053-54). The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).	Buffers (non-ZB). within this range are confirmed as prior art by the '686 patent. (Ex.1001-0016; Ex. 1004, ¶ 109, page 1004-0053); (Ex. 1004 ¶ 109, page 1004-0053-54). The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).

To a POSITA, the invention of claim 7 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it only

uses a prior art buffer concentration previously used in CE, with no change in the prior art function that the ZB had been known to perform and no change in the prior art result attributable to the ZB.

Claim 10	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1. (Ex. 1004, ¶110, page 0054).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004, ¶ 110, page 1004-0054).
said capillary tube is naked.	Shihabi teaches tubes as being washed and rinsed.(Ex.1011-0001; Ex. 1004 ¶110, page 1004-0054). Coufal describes the tubes as bare silica. (Ex.1013-0001; Ex. 1004 ¶110, page 1004-0054). The '686 patent confirms that naked tubes	Coufal teaches bare silica tubes. (Ex.1013-0001; Ex. 1004, ¶110, page 1004-0054). The '686 patent confirms that naked tubes are prior art. (Ex. 1001-0016 (col. 1, lines 36-38). and 1001-1018 (col. 5,

Claim 10	Shihabi in view of Coufal	Huang in view of Coufal
	are prior art. (Ex. 1001-0016 (col. 1, lines 36-38). and 1001-1018 (col. 5, lines 6-15; Ex. 1004, ¶110, page 1005-0054-55). The use of naked capillary tube would have been obvious to a POSITA. (Ex. 1004, ¶110, page 1004-0055).	lines 6-15; Ex. 1004, ¶110, page 1004-0054). The use of naked capillary tube would have been obvious to a POSITA. (Ex. 1004, ¶110, page 1004-0055).

To a POSITA, the invention of claim 10 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it uses a well-known prior art form of capillary tube with no change in the function that the prior art tube had been known to perform and no change in the prior art result attributable to the tube.

Claim 11	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1. (Ex. 1004. ¶ 111, page 1004-0055).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004. ¶ 111, page 1004-0055).
said capillary tube is formed from fused silica.	Coufal teaches that the tube “can also” be coated (Ex. 1013-0001;Ex. 1004. ¶ 111, page 1004-0055). The ‘686 patent refers to fused silica capillaries as prior art. (Ex. 1001-0002; Ex.1001-0018, col. 5, lines 6-15;Ex. 1004. ¶ 111, page 1004-0055). The use of a fused silica capillary tube would have been obvious to a POSTA.	Huang disclose fused silica capillaries. (Ex. 1015- page 0001; Ex. 1004. ¶ 111, page 1004-0055).). Coufal teaches that the tube “can also” be coated (Ex. 1013-0001; Ex. 1004. ¶ 111, page 1004-0055). The ‘686 patent refers to fused silica capillaries as prior art. (Ex. 1001-0002; Ex.1001-0018, col. 5, lines 6-15; Ex. 1004. ¶ 111, page 1004-0055-56).

Claim 11	Shihabi in view of Coufal	Huang in view of Coufal
	(Ex. 1004. ¶ 111, page 1004-0056).	The use of a fused silica capillary tube would have been obvious to a POSITA(Ex. 1004. ¶ 111, page 1004-0056).

To a POSITA, the invention of claim 11 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it uses a well-known prior art form of capillary tube with no change in the function that the prior art tube had been known to perform and no change in the prior art result attributable to the tube.

Claim 12	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1. (Ex. 1004, ¶ 112, page 1004-0056).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004, ¶ 112, page 1004-0056).

Claim 12	Shihabi in view of Coufal	Huang in view of Coufal
said analysis buffer solution has a pH from about 8 to about 10.	Shihabi teaches a pH of 8.5 (Ex. 1011-0001; Ex.1004 ¶ 112, page 1004-0056). Coufal teaches analysis buffers with a pH from 6.8 to 9.5 (Ex. 1013-1008, Ex.1004 ¶ 112, page 1004-0056). It would have been obvious to a POSITA to use a pH range of 8 to 10 for the analysis buffer. (Ex.1004 ¶ 112, page 1004-0056-57).	Huang teaches prior art analysis buffers with pH of 8.6 (among others). (Ex.1015-0001; Ex.1004 ¶ 112, page 1004-0056). Coufal teaches analysis buffers with a pH from 6.8 to 9.5 (Ex. 1013-0008). It would have been obvious to a POSITA to use a pH range of 8 to 10 for the analysis buffer.(Ex. 1004 ¶112, page 1004-0057).

To a POSITA, the invention of claim 12 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it uses a well-known prior art range of buffer pH previously used in CE, with no change in

the prior art function of the buffer pH range and no change in the prior art result attributable to the pH range.

Claim 13	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the above chart for this GROUND 1 for claim 1. (Ex.1004 ¶113, page 1004-0058).	See the above chart for this GROUND 1 for claim 1. (Ex. 1004 ¶ 113, page 1004-0058).
said blood sample is selected from the group consisting of normal blood, abnormal blood, washed blood, decanted blood, centrifuged blood, haemolyzed blood, and whole blood.	Shihabi teaches hemolysis of the sample. (Ex.1011-0001; Ex. 1004 ¶ 113, page 1004-0057). It would have been obvious to a POSITA that blood samples for capillary electrophoresis are necessarily normal or abnormal – that is the logical reason to perform CE on the sample. (Ex.	It would have been obvious to a POSITA that blood samples for capillary electrophoresis are necessarily normal or abnormal – that is the logical reason to perform CE on the sample. (Ex. 1004 ¶113, page 1004-0057-0058). The ‘686 patent confirms that CE is used in the prior

Claim 13	Shihabi in view of Coufal	Huang in view of Coufal
	1004 ¶113, page 1004-0057-58). The ‘686 patent confirms that CE is used in the prior art to detect abnormal hemoglobins. (Ex. 1001-0002; Ex. 1004 ¶113, page 1004-0058).	art to detect abnormal hemoglobins. (Ex. 1001-0002; Ex. 1004 ¶113, page 1004- 0058-59).

To a POSITA, the invention of claim 13 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it was known in the prior art to use CE on normal and/or abnormal blood samples with no change in the prior art function of CE on blood and no change in the prior art result, namely, quantifying the variants to determine if the blood was normal or abnormal.

Claim 14	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 13, wherein	See the immediately preceding chart for the entirety of claim 13. (Ex.1004 ¶114 page 1004-0059).	See the immediately preceding chart for the entirety of claim 13. (Ex.1004 ¶114 page 1004-0059).
said blood sample is a sample of haemolyzed blood.	Shihabi teaches hemolysis of the sample. (Ex.1011-0001). (Ex.1004 ¶114 page 1004-0059).	It would have been obvious to a POSITA that blood samples for capillary electrophoresis can be hemolyzed. (Ex.1004 ¶114 page 1004-0059).

To a POSITA, the invention of claim 14 would have been obvious as of the invention date for the reason explained above as to claims 1 and 13 and because it was known in the prior art to use CE on hemolyzed blood with no change in the prior art function attributable to hemolyzed blood and no change in the result attributable to hemolyzed blood, namely, quantifying the variants in the blood to determine if the blood was normal or abnormal.

Claim 17	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the first chart for this GROUND 1 for claim 1. (Ex. 1004, ¶115, page 1004-0059).	See the first chart for this GROUND 1 for claim 1. (Ex. 1004, ¶115, page 1004-0059).
said analysis buffer solution further comprises a pH modifier.	Shihabi teaches adjusting the pH. (Ex. 1011-0001). (Ex. 1004, ¶115, page 1004-0060). Coufal teaches adjusting the pH of the Tris buffer (Ex. 1013-0002, Table 1). (Ex. 1004, ¶115, page 1004-0060). Even first year undergraduate students are taught how to adjust pH using sodium	Huang teaches adjusting the pH of the running buffer (Ex. 1015-0002). (Ex. 1004, ¶115, page 1004-0060). Coufal teaches adjusting the pH of the Tris buffer (Ex. 1013-0002, Table 1). (Ex. 1004, ¶115, page 1004-0060). Even first year undergraduate students are taught how to adjust pH using sodium

Claim 17	Shihabi in view of Coufal	Huang in view of Coufal
	hydroxide.(Ex. 1004 ¶ 74, page 1004-0036). It would have been obvious to a POSITA that the pH of the buffer solution can be modified or adjusted. (Ex. 1004 ¶ 116, page 1004-0060).	hydroxide.(Ex. 1004 ¶ 74, page 1004-0036). It would have been obvious to a POSITA that the pH of the buffer solution can be modified or adjusted. (Ex. 1004 ¶ 116, page 1004-0060).

To a POSITA, the invention of claim 17 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it was well-known in the prior art to **modify or adjust the pH** of a solution to reach a desired pH or pH range.

Claim 18	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 17, wherein	See the immediately preceding chart for claim 17. (Ex. 1004 ¶116, page 1004-0060).	See the immediately preceding chart for claim 17.(Ex.1004 ¶116, page 1004-0060).

Claim 18	Shihabi in view of Coufal	Huang in view of Coufal
said pH modifier is selected from the group consisting of lithium hydroxide, sodium hydroxide, potassium, hydroxide, rubidium hydroxide, caesium hydroxide, francium hydroxide, mono-alkylammonium hydroxide, di-alkylanimonium hydroxide, tri-alkylammonium hydroxide and tetra-alkylammonium hydroxide said alkylammonium hydroxide containing 1	Coufal teaches the use of sodium hydroxide. (Ex 1013-0002); (Ex. 1004 ¶116, page 1040-0061). Even first year undergraduate students are taught how to adjust pH using sodium hydroxide.(Ex. 1004 ¶ 74, page 1004-0036). It would have been obvious to a POSITA that sodium hydroxide (NaOH) is used to modify pH. (Ex. 1004 ¶116 page 1004-0061).	Coufal teaches the use of sodium hydroxide. (Ex 1013-0002); (Ex 1004 ¶116, page 1040-0061). Even first year undergraduate students are taught how to adjust pH using sodium hydroxide. (Ex. 1004 ¶ 74, page 1004-0036). It would have been obvious to a POSITA that sodium hydroxide (NaOH) is used to modify pH. (Ex. 1004 ¶116 page 1004-0061).

Claim 18	Shihabi in view of Coufal	Huang in view of Coufal
to 8 carbon atoms in the alkyl portion.		

To a POSITA, the invention of claim 18 would have been obvious as of the invention date for the reason explained above as to claims 1 and 17, because at least one of the pH modifiers listed in the claim (sodium hydroxide) is a well-known prior art pH modifier, used to in the prior art to modify or adjust the pH of a solution to reach a desired pH or pH range, with no change in the function of sodium hydroxide and no change in the prior art result of using sodium hydroxide as a pH modifier.

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
A free solution CE method for separating and resolving haemoglobins in a biological sample by CE, said biological	Shihabi teaches everything in the preamble of the claim including hemolysis of the sample. (Ex.1011-0001; Ex. 1004 ¶118, page 1004-0062).	Huang teaches everything in the preamble of the claim except the specific nature of the sample. (Ex.1011-0001; Ex. 1004 ¶118, page 1004-0062).

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
sample being a blood sample, said sample being selected from the group consisting of normal blood, abnormal blood, washed blood, decanted blood, centrifuged blood, whole blood and haemolyzed blood, said method comprising:	The ‘686 patent confirms that CE is used in the prior art to detect abnormal hemoglobins. (Ex. 1001-0002; Ex. 1004 ¶118, page 1004-0062).	It would have been obvious to a POSITA that blood samples for capillary electrophoresis are necessarily normal or abnormal – that is the logical reason to perform CE on the sample. (Ex. 1004 ¶118, page 1004-0062-63). The ‘686 patent confirms that CE is used in the prior art to detect abnormal hemoglobins. (Ex. 1001-0002; Ex. 1004 ¶118, page 1004-0063).

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
introducing said biological sample into a capillary tube comprising a solution of an analysis buffer, wherein said analysis buffer is of the ZB type and at least one FI, and	Shihabi teaches everything in the first step of the method including use of an arginine-Tris buffer (Tris is a ZB), but Shihabi does not teach the use of the FI. (Ex.1011-0001; Ex. 1004 ¶118, page 1004-0064). Coufal teaches use, in capillary columns, of a solution including a ZB (Tris). and including at least one flow inhibitor (ethylenediamine) and that another FI (SDS) provided a 10% EOF reduction and that other buffer - FI	Huang teaches everything in the first step of the method including the use of a ZB (bis-tris propane). and that “reducing both the EOF and the electrophoretic mobilities of the two species can maximize the resolution (at the expense of longer separation time” (Ex. 1015-0001) but does teach the use of the FI. (Ex.1011-0001; Ex. 1004 ¶118, page 1004-0064).

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
	combinations provided a 20% reduction. (Ex. 1013-0001 (Summary), and 1013-0009; Ex. 1004 ¶118, page 1004-0064-65).	Coufal teaches use, in capillary columns, of a solution including a ZB buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that another FI (SDS) provided a 10% EOF reduction and other buffer – FI combinations provided a 20% reduction. (Ex. 1013-0001 (Summary), and 1013-0009; Ex. 1004 ¶118, page 1004-0064-65). .
electrophoresing said biological sample in said analysis buffer and at least one said FI,	Shihabi teaches the step of CE resulting in separation by migration (Ex.1011-	Huang teaches that electrophoresing involves migration of the hemoglobin variants. (Ex.

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
whereby said haemoglobins are separated by migration.	0003; Ex. 1004 ¶118, page 1004-0065-66). Coufal teaches the use of both ZB and FI and refers to the migration time. (Ex. 1013-0002; Ex. 1004 ¶118, page 1004-0066). It would have been obvious to a POSITA that CE causes components of the sample to be separated by migration. (Ex. 1004 ¶118, page 1004-0066).	1015-0001; Ex 1004 ¶118, page 1004-0065-66). Coufal teaches the use of both ZB and FI and refers to the migration time. (Ex. 1013-0002).(Ex. 1004 ¶118, page 1004-0066). It would have been obvious to a POSITA that CE causes components of the sample to be separated by migration. (Ex. 1004 ¶118, page 1004-0066).

To a POSITA, the invention of claim 23 would have been obvious as of the invention date because it only uses familiar **ZB** and **FI** buffer components previously used in prior art CE, and with no change in their respective functions, that they had been known to perform and providing the predictable result of

reducing EOF thus providing better separation of the components of the sample by migration when used in hemoglobin CE.

B. GROUND 2: Claims 15 and 16 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011). or Huang (Ex. 1015). each in view of Coufal (Ex. 1013). and Camilleri (Ex. 1020).(Ex. 1004 ¶ 119, page 1004-0066).

These two dependent claims depend (directly or indirectly) upon claim 1 but are addressed separately as they include **ZB** commonly used in CE but not disclosed in Shihabi or Huang or Coufal. Rather, they are disclosed in prior art documents such as Camilleri. (Id.).

Accordingly, the claims in this GROUND 2 would have been obvious under 35 U.S.C. §103 to a POSITA prior to the invention date as explained in the following claim charts. (Ex. 1004 ¶ 121, page 1004-0067).

Claim 15	Shihabi in view of Coufal and Camilleri	Huang in view of Coufal and Camilleri
The method according to claim 1, wherein	See the first chart for GROUND 1 (Section VII.	See the first chart for GROUND 1 (Section VII.

Claim 15	Shihabi in view of Coufal and Camilleri	Huang in view of Coufal and Camilleri
	A.). for claim 1.(Ex. 1004 ¶ 122, page 1004-0067).	A.). for claim 1. (Ex. 1004 ¶ 122, page 1004-0067).
said analysis buffer is selected from tricine , TAPS, TABS, AMPSO, bicine or HEPBS, or an association of Tris, AMPD or bis Tris propane with an amino acid.	Camilleri teaches selecting the ZB from tricine among other ZB for CE. (Ex.1020-0002; Ex. 1004 ¶ 122, page 1004-0067).	Camilleri teaches selecting the ZB from tricine among other ZB for CE. (Ex.1020-0002; Ex. 1004 ¶ 122, page 1004-0067).

To a POSITA, the invention of claim 15 would have been obvious as of the invention date for the reason explained above as to claim 1 and because at least one **ZB** listed in the claim was a well-known prior art **ZB** used in CE, and there is no change in the function of the **ZB**.

Claim 16	Shihabi in view of Coufal and Camilleri	Huang in view of Coufal and Camilleri
The method according to claim 15, wherein	See the immediately preceding chart for claim 15. (Ex. 1004 ¶ 123; page 1004-0068).	See the immediately preceding chart for claim 15. (Ex. 1004 ¶123, page 1004-0068).
said analysis buffer is tricine .	Camilleri teaches selecting the ZB from tricine among other ZB for CE. (Ex.1020-0002). (Ex. 1004 ¶ 123, page 1004-0068).	Camilleri teaches selecting the ZB from tricine among other ZB for CE. (Ex.1020-0002).(Ex. 1004 ¶ 123, page 1004-0068).

To a POSITA, the invention of claim 16 would have been obvious as of the invention date for the reason explained above as to claims 1 and 15, and because the **ZB** listed in the claim was a well-known prior art **ZB** used in CE with no change in the function of the **ZB**.

C. GROUND 3: Claims 4, 5, 8, 9, 19-22 and 24 would have been obvious to a POSITA at the time of the invention over either of Shihabi (Ex. 1011) or Huang (Ex. 1015) each in view of Coufal (Ex. 1013) and Landers (Ex. 1026).

(Ex. 1004 ¶ 124; page 1004-0068-69).

These claims address details of the **FI** commonly used in CE but not disclosed in Shihabi or Huang or Coufal. Rather, they are disclosed prior art documents such as Landers. (Ex. 1004 ¶ 125, page 1004-0069).

Accordingly, the claims in this GROUND 3 would have been obvious under 35 U.S.C. §103 to a POSITA prior to the invention date as explained in the following claim charts. (Ex. 1004 ¶ 126, page 1004-0069).

Claim 4	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 1, wherein	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 126; page 1004-0069).	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 126; page 1004-00690).
said at least one FI is of an aliphatic or a cyclic diamine or a polyamine type.	Coufal teaches the combination of Tris (ZB) . and ethylenediamine (FI) . to reduce EOF(Ex. 1013-0001). Ethylenediamine is	Coufal teaches the combination of Tris (ZB) . and ethylenediamine (FI) . to reduce EOF(Ex. 1013-0001). Ethylenediamine is

Claim 4	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	an aliphatic diamine. (Ex. 1004 ¶ 126, page 1004-0069-70). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). Ex. 1004 ¶ 126, page 1004-0070). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶ 126, page 1004-0070).	an aliphatic diamine. Ex. 1004 ¶ 126, page 1004-0069-70). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). Ex. 1004 ¶ 126, page 1004-0070). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶ 126, page 1004-0070).

To a POSITA, the invention of claim 4 would have been obvious as of the invention date for the reason explained above as to claim 1 and because at least one **FI** from among those listed in the claim was a well-known prior art **FI** used in CE with no change in the function of the **FI**.

Claim 5	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 1, wherein	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 127; page 1004-0071).	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 127; page 1004-0071).
said at least one FI is selected from the group consisting of aliphatic diamines , aliphatic polyamines, their acceptable derivatives and salts, and mixtures thereof.	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) . to reduce EOF(Ex. 1013-0001). Ethylenediamine is an aliphatic diamine. (Ex. 1004 ¶ 127, page 1004-0071).	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) . to reduce EOF(Ex. 1013-0001). Ethylenediamine is an aliphatic diamine. (Ex. 1004 ¶ 127, page 1004-0071).

Claim 5	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB).(Ex. 1004 ¶127, page 1004-0071). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶ 127, page 1004-0072).	Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 127, page 1004-0071). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶ 127, page 1004-0072).

To a POSITA, the invention of claim 5 would have been obvious as of the invention date for the reason explained above as to claim 1 and because at least one **FI** from among those listed in the claim was a well-known prior art **FI** used in CE

with no change in the function of the **FI**.

Claim 8	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 1, wherein	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 128, page 1004-0072).	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 128, page 1004-0072).
said at least one FI has a concentration from about 0.01 mM to about 50 mM in said analysis buffer solution.	Coufal teaches the combination of Tris (ZB). and ethylenediamine (FI). to reduce EOF(Ex. 1013-0001). Ethylenediamine is an aliphatic diamine. (Ex. 1004 ¶ 128, pages 1004-0071-72). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and	Coufal teaches the combination of Tris (ZB). and ethylenediamine (FI). to reduce EOF(Ex. 1013-0001). Ethylenediamine is an aliphatic diamine. (Ex. 1004 ¶ 128, pages 1004-0071-72). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and

	confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 128, page 1004-0072-73) The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶128, page 1004-0073). Landers teaches the DAB concentration of 250 µM (Ex. 1026-0003) which is 0.250 mM within the range of the claim. (Id.) The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).	confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB).(Ex. 1004 ¶ 128, page 1004-0072-73)/ The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶128, page 1004-0073). Landers teaches the DAB concentration of 250 µM (Ex. 1026-0003) which is 0.250 mM within the range of the claim. (Id.) The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).
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To a POSITA, the invention of claim 8 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it was well-known to use a prior art FI buffer concentration range that is within the range of the claim, and there does not appear to be any criticality of the range and/or its end points. (Ex. 1004 ¶128, page 1004-0074).

Claim 9	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 1, wherein	See the first chart for GROUND 1 (Section VII. A.). for claim 1. (Ex. 1004 ¶129; page 1004-0074)	See the first chart for GROUND 1 (Section VII. A.). for claim 1. (Ex. 1004 ¶ 129, page 1004-0074).
said at least one FI has a concentration from about 0.10 mM to about 20 mM in said analysis buffer solution.	Coufal teaches the combination of Tris (ZB). and ethylenediamine (FI). to reduce EOF(Ex. 1013-0001). Ethylenediamine is an aliphatic diamine. (Ex.	Coufal teaches the combination of Tris (ZB). and ethylenediamine (FI). to reduce EOF(Ex. 1013-0001). Ethylenediamine is an aliphatic diamine. (Ex.

	1004 ¶129, page 1004-0074-75). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001). and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB) (Ex. 1004 ¶129, page 1004-0075). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶129, page 1004-0075). Landers teaches the DAB concentration of 250 µM (Ex. 1026-0003) which is 0.250 mM within the	1004 ¶129, page 1004-0074-75). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001). and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶129, page 1004-0075). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶129, page 1004-0075). Landers teaches the DAB concentration of 250 µM (Ex. 1026-0003) which is .250 mM within the
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	range of the claim. (Ex. 1004 ¶129, page 1004-0075-76). The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).	range of the claim. (Ex. 1004 ¶129, page 1004-0075-76). The range is thus <i>prima facie</i> obvious (M.P.E.P. §2144.05 and cases cited therein).
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To a POSITA, the invention of claim 9 would have been obvious as of the invention date for the reason explained above as to claim 1 and because it was well-known to use a prior art FI buffer concentration that is within the range in the claim, and there does not appear to be any criticality of the range and/or its end points. (Ex. 1004 ¶129, page 1004-0076).

Claim 19	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 1, wherein	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 130, page 1004-0076).	See the first chart for GROUND 1 (Section VII. A.) for claim 1. (Ex. 1004 ¶ 130, page 1004-0076).

said an (sic. “at”). least one FI is selected from the group consisting of 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, diethylenetriamine, spermine, N,N'-dimethyl-1,6-hexanediamine, tetraethylenepentamine, N,N,N',N'-tetramethyl-1,4-butanediamine, their acceptable derivatives and salts, and mixtures thereof.	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001). (Ex. 1004 ¶ 130, page 1004-0076). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001). and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 130, page 1004-0076-77).	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001). (Ex. 1004 ¶ 130, page 1004-0076). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001). and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 130, page 1004-0077).
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To a POSITA, the invention of claim 19 would have been obvious as of the

invention date for the reason explained above as to claim 1 and because it was well-known to use a prior art **FI** from among those prior art **FIs** listed in the claim with no change in function of the **FI**.

Claim 20	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 19, wherein	See the immediately preceding chart for claim 19.(Ex. 1004 ¶ 131, page 1004-0077).	See the immediately preceding chart for claim 19. (Ex. 1004 ¶ 131, page 1004-0077).
said at least one FI is selected from the group consisting of 1,4-diaminobutane , 1, 5-diaminopentane, 1,6-diaminohexane, diethylenetriamine, and N,N,N',N'-tetramethyl-1,4-butanediamine, their acceptable	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001; Ex. 1004 ¶ 131, page 1004-0077-78). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001; Ex. 1004 ¶ 131, page 1004-0077-78). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is

derivatives and salts, and mixtures thereof.	1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 131, page 1004- 0078).	1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 131, page 1004- 0078).
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To a POSITA, the invention of claim 20 would have been obvious as of the invention date for the reason explained above as to claims 1 and 19, and because it was well-known to use a prior art **FI** from among those prior art **FIs** listed in the claim with no change in function of the **FI**.

Claim 21	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
The method according to claim 20, wherein	See the immediately preceding chart for claim 20. (Ex. 1004 ¶132, page 1004-0078).	See the immediately preceding chart for claim 20. (Ex. 1004 132. page 1004-0078).
said at least one FI is 1,4-diaminobutane .	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI)	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI)

	to reduce EOF(Ex. 1013-0001; Ex. 1004 ¶ 132, page 1004-0079). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 132, page 1004-0079).	to reduce EOF(Ex. 1013-0001; Ex. 1004 ¶ 132, page 1004-079). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 132, page 1004-0079).
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To a POSITA, the invention of claim 21 would have been obvious as of the invention date for the reason explained above as to claims 1 and 20, and because it was well-known to use a prior art FI from among those prior art FIs listed in the claim with no change in function of the FI.

Claim 22	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
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The method according to claim 21, wherein	See the immediately preceding chart for claim 21.(Ex. 1004 ¶ 133, page 1004-0080).	See the immediately preceding chart for claim 21. (Ex. 1004 ¶ 133, page 1004-0080).
said at least one FI is 1,4-diaminobutane in the hydrochloride form.	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001).(Ex. 1004 ¶ 133, page 1004-0080). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). Ex. 1004 ¶ 133, page 1004-0080). It would have been obvious to a POSITA to	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001. Ex. 1004 ¶ 133, page 1004-0080). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). Ex. 1004 ¶ 133, page 1004-0080). It would have been obvious to a POSITA to use

	use chemicals in various forms such as salt and/or hydrochloride. (Ex. 1004 ¶ 133, page 1004-0080).	chemicals in various forms such as salt and/or hydrochloride. (Ex. 1004 ¶ 133, page 1004-0080).
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To a POSITA, the invention of claim 22 would have been obvious as of the invention date for the reason explained above as to claims 1 and 21, and because it was well-known to use chemicals in various forms such as salt and/or hydrochloride and no different function is disclosed when using an FI in a salt and/or hydrochloride form.

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
A free solution CE method for separating and resolving haemoglobins in a biological sample by CE, said biological	Shihabi teaches everything in the preamble of the claim. (Ex. 1004 ¶ 134, page 1004-0081).	Huang teaches everything in the preamble of the claim. (Ex. 1004 ¶ 134, page 1004-0081).

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
sample being a blood sample, said method comprising		
introducing said biological sample into a capillary tube comprising a solution of an analysis buffer, wherein said analysis buffer is of the zwitterionic type and at least one FI , said FI being an aliphatic or a cyclic diamine or a polyamine type, and	Shihabi teaches everything in the first step of the method including use of an arginine-Tris buffer (Tris is a ZB), but Shihabi does not teach the use of the FI. (Ex. 1004 ¶ 134, pages 1004-0081-82). Coufal teaches use, in capillary columns, of a solution including a ZB buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that another FI (SDS) provided	Huang teaches everything in the first step of the method including the use of a ZB (bis-tris propane) and that “reducing both the EOF and the electrophoretic mobilities of the two species can maximize the resolution (at the expense of longer separation time” (Ex. 1015-0001 0001). but does teach the use of the FI. (Ex. 1004 ¶ 134, page 1004-0081-82).

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013-0001 (Summary) and 1013-0009). Ethylenediamine is an aliphatic diamine. (Ex. 1004 ¶ 134, pages 1004-0082-83). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB).(Ex.	Coufal teaches use, in capillary columns, of a solution including a ZB buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that another FI (SDS) provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013-0001 (Summary) and 1013-0009) Ethylenediamine is an aliphatic diamine. (Ex.

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	1004 ¶ 134., page 1004-0083). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶134, page 1004-0083).	1004 ¶ 134, pages 1004-0082-83). Landers teaches use of putrecine as the FI in CE (Ex.1026-0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026-0002). (DAB). (Ex. 1004 ¶ 134, page 1004-0083). The ‘686 patent confirms that DAB is an “aliphatic or a cyclic diamine or a polyamine” (Ex. 1001-0017; Ex. 1004 ¶ 134, page 1004-0084).
electrophoresing said biological sample in	Shihabi teaches the step of CE resulting in separation	Huang teaches that electrophoresing involves

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
said analysis buffer and at least one said flow inhibitor, whereby said haemoglobins are separated by migration.	by migration (Ex.1011-00031; Ex. 1004 ¶ 134, page 1004-0084). Coufal teaches the use of both ZB and FI and refers to the migration time (Ex. 1013-0002; Ex. 1004 ¶ 134, page 1004-0084). It would have been obvious to a POSITA that capillary electrophoresis causes components of the sample to be separated by migration. (Ex. 1004 ¶134, page 1004-0085).	migration of the hemoglobin variants. (Ex. 1015-0001; Ex. 1004 ¶ 134, page 1004-0083-84). Coufal teaches the use of both ZB and FI and refers to the migration time. (Ex. 1013-0002; Ex.1004 ¶134, page 1004-84). It would have been obvious to a POSITA that capillary electrophoresis causes components of the sample to be separated by migration. (Ex. 1004 ¶134, page 1004-0085).

To a POSITA, the invention of claim 24 would have been obvious as of the

invention date because it only uses familiar ZB and FI buffer components previously used in CE, and with no change in their respective functions that they had been known to perform and providing the predictable result of reducing EOF thus providing better separation of the components of the sample by migration when used in hemoglobin CE.

VIII. CONCLUSION

Based on the arguments and prior art references disclosed and discussed in this Petition, the challenged claims of the '686 Patent would have been obvious to a POSITA at the time of the invention.

This Petition relies primarily on prior art that was not before the Examiner or that was not applied by the Examiner. The prior art establishes a reasonable likelihood that Petitioner will prevail on at least one claim.

Petitioner therefore request institution of an *inter partes* review to cancel the challenged claims of the '686 Patent.

Dated: April 11, 2024.

Respectfully submitted,

/ JIS/

Jerold I. Schneider

Reg. No. 24,765

Schneider IP Law

21048 95th Ave S. Suite D-206

Boca Raton, FL 33428-1567

Phone: 561-309-5374

jerold.schneider@schneideriplaw.legal

Attorney for Petitioner

Certificate of Compliance with Word Count

Pursuant to 37 C.F.R. § 42.24(d), I certify that this Petition complies with the type-volume limits of 37 C.F.R. § 42.24(a).(l).(i) because it contains 11,966 words, according to the word-processing system used to prepare this petition, excluding the parts of this petition that are exempted by 37 C.F.R. § 42.24(a), namely Table of Contents, Table of Authorities, Mandatory Notices under 37 C.F.R. § 42.8, Certificate of Service. Certificate of Word Count, and List of Exhibits.

Dated: April 11, 2024.

 / JIS/
Jerold I. Schneider
Reg. No. 24,765
Schneider IP Law
21048 95th Ave S. Suite D-206
Boca Raton, FL 33428-1567
Phone: 561-309-5374

jerold.schneider@schneideriplaw.legal

Attorney for Petitioner

Certificate of Service

I hereby certify that on this 11th day of April, 2024, I caused a true and correct copy of this PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 7,887,686, together with Exhibits 1001 through and including Exhibit 1026, to be served by Priority Mail Express® or by means at least as fast and reliable as Priority Mail Express® pursuant to 37 C.F.R. §42.105, to the attorneys of record for U.S. Patent No. 7,887,686, at their current address:

LERNER DAVID LITTENBERG KRUMHOLZ & MENTLIK

LERNERDAVID LLP
20 Commerce Drive
Cranford, N.J. 07016

Dated: April 11, 2024.

Respectfully submitted,

/ JIS/
Jerold I. Schneider
Reg. No. 24,765
Schneider IP Law
21048 95th Ave S. Suite D-206
Boca Raton, FL 33428-1567
Phone: 561-309-5374

jerold.schneider@schneideriplaw.legal

Attorney for Petitioner

APPENDIX - CLAIM LISTING

1. A free solution capillary electrophoresis method for separating and resolving haemoglobins in a biological sample by capillary electrophoresis, said biological sample being a blood sample, said method comprising:

introducing said biological sample into a capillary tube comprising a solution of an analysis buffer, wherein said analysis buffer is of the zwitterionic type and at least one flow inhibitor, and

electrophoresing said biological sample in said analysis buffer and at least one said flow inhibitor, whereby said haemoglobins are separated by migration, and wherein said haemoglobins are a haemoglobin HbA2 and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin.

2. The method according to claim 1, further comprising detecting said haemoglobins.

3. The method according to claim 1, wherein the zwitterionic buffer is formed by one or two molecules and comprises at least one amine function, at least one acid function and at least one hydroxyl function in the position opposite to the acid function.

4. The method according to claim 1, wherein said at least one flow inhibitor is of an aliphatic or a cyclic diamine or a polyamine type.

5. The method according to claim 1, wherein said at least one flow inhibitor is selected from the group consisting of aliphatic diamines, aliphatic polyamines, their acceptable derivatives and salts, and mixtures thereof.

6. The method according claim 1, wherein said analysis buffer has a concentration in said analysis buffer solution from about 20 mM to about 500 mM.

7. The method according to claim 1, wherein said analysis buffer has a concentration in said analysis buffer solution from about 100 mM to about 250 mM.

8. The method according to claim 1, wherein said at least one flow inhibitor has a concentration from about 0.01 mM to about 50 mM in said analysis buffer solution.

9. The method according to claim 1, wherein said at least one flow inhibitor

has a concentration from about 0.10 mM to about 20 mM in said analysis buffer solution.

10. The method according to claim 1, wherein said capillary tube is naked.

11. The method according to claim 1, wherein said capillary tube is formed from fused silica.

12. The method according to claim 1, wherein said analysis buffer solution has a pH from about 8 to about 10.

13. The method according to claim 1, wherein said blood sample is selected from the group consisting of normal blood, abnormal blood, washed blood, decanted blood, centrifuged blood haemolyzed blood, and whole blood.

14. The method according to claim 13, wherein said blood sample is a sample of haemolyzed blood.

15. The method according to claim 1, wherein said analysis buffer is selected from tricine, TAPS, TABS, AMPSO, bicine or HEPBS, or an association

of Tris, AMPD or bis Tris propane with an amino acid.

16. The method according to claim 15, wherein said analysis buffer is tricine.

17. The method according to claim 1, wherein said analysis buffer solution further comprises a pH modifier.

18. The method according to claim 17 wherein said pH modifier is selected from the group consisting of lithium hydroxide, sodium hydroxide, potassium, hydroxide, rubidium hydroxide, caesium hydroxide, francium hydroxide, mono-alkylammonium hydroxide, di-alkylammonium hydroxide, tri-alkylammonium hydroxide and tetra-alkylammonium hydroxide said alkylammonium hydroxide containing 1 to 8 carbon atoms in the alkyl portion.

19. The method according to claim 1, wherein said at least one flow inhibitor is selected from the group consisting of 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, diethylenetriamine, spermine, N,N'-dimethyl-1,6-hexanediamine, tetraethylenepentamine, N,N,N',N'-tetramethyl-1,4-butanediamine, their acceptable derivatives and salts, and mixtures

thereof.

20. The method according to claim 19, wherein said at least one flow inhibitor is selected from the group consisting of 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, diethylenetriamine and N,N,N',N'-tetramethyl-1,4-butanediamine, their acceptable derivatives and salts, and mixtures thereof.

21. The method according to claim 20, wherein said at least one flow inhibitor is 1,4-diaminobutane.

22. The method according to claim 21, wherein said at least one flow inhibitor is 1,4-diaminobutane in the hydrochloride form.

23. A free solution capillary electrophoresis method for separating and resolving haemoglobins in a biological sample by capillary electrophoresis, said biological sample being a blood sample, said sample being selected from the group consisting of normal blood, abnormal blood, washed blood, decanted blood, centrifuged blood, whole blood and haemo-lyzed blood, said method comprising:
introducing said biological sample into a capillary tube comprising a

solution of an analysis buffer, wherein said analysis buffer is of the zwitterionic type and at least one flow inhibitor, and

electrophoresing said biological sample in said analysis buffer and at least one said flow inhibitor, whereby said haemoglobins are separated by migration.

24. A free solution capillary electrophoresis method for separating and resolving haemoglobins in a biological sample by capillary electrophoresis, said biological sample being a blood sample, said method comprising:

introducing said biological sample into a capillary tube comprising a solution of an analysis buffer, wherein said analysis buffer is of the zwitterionic type and at least one flow inhibitor, said flow inhibitor being an aliphatic or a cyclic diamine or a polyamine type, and

electrophoresing said biological sample in said analysis buffer and at least one said flow inhibitor, whereby said haemoglobins are separated by migration.