

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HELENA LABORATORIES CORPORATION

Petitioner,

v.

SEBIA

Patent Owner

Case No.:

DECLARATION OF ALAN H.B. WU, Ph.D.

1. I, Alan H.B. Wu, Ph.D., hereby declare as follows:

I. Engagement and compensation.

2. I have been retained by Jerold I. Schneider, P.A. d/b/a Schneider IP Law, to serve as an expert in this *inter partes* review proceeding. I have been asked to provide my technical review, analysis, insights, and opinions regarding the references that form the basis for the grounds of unpatentability set forth in the Petition for *inter partes* review of U.S. Patent No. 7,887,686 (the '686 Patent) (Ex.1001). For this service, my billing rate is an hourly consulting fee of \$400/hour, and I am reimbursed for actual expenses. My compensation in no way depends on the outcome of this matter.

II. Summary of opinions.

3. To minimize repetition, the following should be considered to be included in all of my discussions about prior art and in all my opinions and conclusions: (1) each item of prior art, to the extent relied upon by me in formulating and expressing my opinions, is in the field of capillary electrophoresis (including subsets of capillary electrophoresis); (2) each item of prior art, to the extent relied upon by me in formulating and expressing my opinions, is

“analogous” to every other item of prior art (to the extent relied upon by me); (3) all opinions relating to two or more items of prior art with the language “in view of” or “evidenced by” reflects how persons of ordinary skill in the art would understand, consider and combine the teachings of the prior art; and (4) the term “prior art” refers to information that was public “at the time of the invention” identified in Section VII. I use the term “analogous” to indicate (a) that the prior art is from the same field of endeavor or interest as addressed in the ‘686 patent, namely hemoglobin variant separation by capillary electrophoresis (even if the prior art solves a different problem, such as other non-hemoglobin separation by capillary electrophoresis, or (b) the prior art is reasonably pertinent to hemoglobin variant separation by capillary electrophoresis and would be considered as such by persons of ordinary skill in the art when seeking to identify, address and solve technical problems, if any. Two non-limiting sources of “analogous” prior art include (a) the prior art presented in the text of the ‘686 patent itself and (b) the prior art submitted to the Patent Office during the proceedings leading up to the grant of the ‘686 patent.

4. My opinion on the relevancy of prior art in the capillary electrophoresis field *per se* is supported by the statement made by Sebia to the Patent Examiner as explained below in Section IX, paragraphs 81 and 82.

5. It is my opinion that claims 1, 2, 3, 6, 7, 10-14, 17, 18 and 23 would have been obvious to a person of ordinary skill in the art at the time of the invention over either Shihabi *et al*, “Hemoglobin A₂ quantification by capillary zone electrophoresis” Electrophoresis (2000) Vol. 21, pages 749-752 (Ex 1011) or Huang et al “The Separation of Hemoglobin Variants by Capillary Zone Electrophoresis,” Journal of High Resolution Chromatography, Vol. 17, pages 675 – 678. (Ex. 1015) each in view of 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”) (Ex. 1013).

6. It is my opinion that claims 15 and 16 would have been obvious to a person of ordinary skill in the art at the time of the invention over either Shihabi *et al*, “Hemoglobin A₂ quantification by capillary zone electrophoresis” Electrophoresis (2000) Vol. 21, pages 749-752 (Ex 1011) or Huang et al “The Separation of Hemoglobin Variants by Capillary Zone Electrophoresis,” Journal of High Resolution Chromatography, Vol. 17, pages 675 – 678. (Ex. 1015) each in view of 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”) and Camilleri “*Capillary Electrophoresis*” 1997, pages 370-371 (Ex. 1020).

7. It is my opinion that claims 4, 5, 8, 9, 19-22 and 24 would have been obvious to a person of ordinary skill in the art at the time of the invention over either Shihabi *et al*, “Hemoglobin A₂ quantification by capillary zone electrophoresis” *Electrophoresis* (2000) Vol. 21, pages 749-752 (Ex 1011) or Huang *et al* “The Separation of Hemoglobin Variants by Capillary Zone Electrophoresis,” *Journal of High Resolution Chromatography*, Vol. 17, pages 675 – 678. (Ex. 1015) each in view of 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”) and Camilleri “*Capillary Electrophoresis*” 1997, pages 370-371 (Ex. 1020) and 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. (Exhibit 1026).

8. Additional opinions that I have arrived at to reach the ultimate opinions of obviousness will appear throughout the present Declaration.

9. I wish to reserve the right to rely on any of the Exhibits to support and/or corroborate my opinions.

III. My Qualifications.

10. Highlighting some of my qualifications, I am currently Chief, Clinical Chemistry and Toxicology Laboratories, San Francisco General Hospital and Professor, Laboratory Medicine, University of California, San Francisco and was formerly Chief Clinical Pharmacogenomics Laboratory, University of California at San Francisco and Co-core Laboratory Director, Zuckerberg San Francisco General Hospital,. As of January 1, 2024, I have stepped down as Editor-in-Chief, *Clinica Chimica Acta*, a position that I held for 23 years. My full *Curriculum Vitae* is attached as Exhibit 1005.

11. My academic credentials include a Bachelor of Science degree in Chemistry from Purdue University and a Ph.D. from the University in Illinois. I can confirm from personal knowledge both as a student and as a teaching professor that the general subject matter of the '686 patent, namely particle separation techniques particle identification (qualitative and quantitative) and equipment for particle separation, is part of analytical chemistry and is a required course for an undergraduate degree in chemistry.

12. I have, among other things, authored and/or co-authored over 500 peer review published articles, received numerous awards around the world, been an invited lecturer around the world, mentored graduate students and supervised postdoctoral fellows as detailed in my CV.

IV. My understanding of the relevant law.

A. Claim construction.

13. I have been asked by counsel to assume that a claim term in an *inter partes* review is to be interpreted by 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. Thus, the claims are to be analyzed in the following manner: First, the language of the claims themselves is of primary importance in the effort to determine precisely what is patented. The terms used in a claim are generally given the ordinary and customary meaning that the terms would have to a person of ordinary skill in the art in question at the time of the effective filing date of the patent claim in question, unless the term is expressly defined in the patent. The person of ordinary skill in the art reads the claim in the context of the entire patent, including the specification. Next, as to the language of the claim terms, the specification is the single best source for interpreting the claim terms. The claim terms may also be interpreted using the record of correspondence between the patent applicant and the Patent Office (prosecution history), and also with reference to other sources of evidence that can help to define the meaning of terms to a person of ordinary skill in the relevant time frame.

B. Obviousness.

14. I have been asked by counsel to assume that a claim in an issued patent can be invalid if it would have been obvious to a person of ordinary skill in the art at the time of the invention of the patent. Obviousness does not require that every element of the claim be in a single prior art reference. Instead, it is possible for claim elements to be described in different prior art references, so long as there is sufficient reason to combine the teachings of the references.

15. I have been asked by counsel to assume that a claim is invalid for obviousness if the differences between the claimed subject matter and the prior art are such that the subject matter as a whole would have been obvious at the time of the invention to a person having ordinary skill in the art to which said subject matter pertains.

16. I have been asked by counsel to assume, therefore, that when evaluating obviousness, one must consider obviousness of the claim “as a whole.” This consideration must be from the perspective of the person of ordinary skill in the relevant art, and that such perspective must be considered as of the time of the invention.

17. I have been informed by counsel that where a patent claim indicates a range (for example, pH or concentration) that that range (a) lies entirely within the range of a prior art document or (b) lies partially within the range of a prior art document and overlaps one end of the range of that prior art document,

and/or (c) lies at one end of the range of that prior art patent, then I may initially consider the claim to be obvious, and I may further conclude obviousness unless the range (in the claim) is critical and achieves some unexpected result relative to the range in the prior art.

18. The information from counsel as stated in the prior paragraph is consistent with my knowledge and experience as it relates to the subject matter of these proceedings. For example, persons of ordinary skill in the art, when considering a range such as pH or concentration, are trained by combined classroom and laboratory courses in undergraduate school, and by their post-baccalaureate research and work experience, if any, how to determine ranges and potential end points in ranges. In that regard, the ranges and end points in capillary electrophoresis, are normally not critical. Workable ranges and end points, regardless of whether they are optimal, may be determined by routine testing.

19. Thus, in the context of the '686 patent, for example, since the prior art described in the patent specification (column 1, lines 49-65) identifies pH values of 8 and 9.98, it is within the ordinary skill of the art to suggest a pH "in the range 8 to 11, preferably in the range 8 to 10" ('686 patent, column 4, lines 33-34) and to claim the pH "about 8 to about 10" (claim 12) even though the experiments reported in the patent only involved pH values of 9.19, 9.37, and 9.40.

20. The level of ordinary skill in the art that I am using for my opinions of the '686 Patent is discussed in Section VI below.

21. The relevant time frame for obviousness, the time of the invention, is discussed in Section VII below.

22. I have been asked by counsel to assume that in considering the obviousness of a claim, one must consider four things. These include the scope and content of the prior art, the level of ordinary skill in the art at the relevant time, the differences between the prior art and the claim, and any “secondary considerations”.

23. I have been asked by counsel to assume all of the following about “secondary considerations.” “Secondary considerations” include real-world evidence that can tend to make a conclusion of obviousness either more probable or less probable. For example, the commercial success of a method embodying a claim of the patent could provide evidence tending to show that the claimed invention would not have been obvious. To understand the strength of the evidence, one would want to know whether the commercial success is traceable to a certain aspect of the claim not disclosed in a single prior art reference (i.e., whether there is a casual “nexus” to the language of a claim). One would also want to know how the market reacted to disclosure of the invention, and whether commercial success might be traceable to things other than innovation, for

example the market power of the seller, an advertising campaign, or the existence of a complex system having many features beyond the claims that might be desirable to a consumer. One would also want to know how the product compared to similar products not embodying the claim. I have been asked by counsel to assume that evidence of commercial success should be reasonably commensurate with the scope of the claim, but that it is not necessary for a commercial product to embody the full scope of the claim.

24. Other kinds of secondary considerations are possible. For example, evidence that the relevant field had a long-established, unsolved problem or need that was later provided for by the claimed invention could be indicative of non-obviousness. Evidence that others had tried, but failed to make an aspect of the claim might indicate that the art lacked the requisite skill to do so. Evidence of copying of the patent owner's products before the patent was published might also indicate that its approach to solving a particular problem would not have been obvious. Evidence that the art recognized the value of products embodying a claim, for example, by praising the named inventors' work, might tend to show that the claim would not have been obvious.

25. I further have been asked by counsel to assume that prior art references can be combined where there is an expressed or implied rationale to do so. Such a rationale might include an expected advantage to be obtained or might

be implied under the circumstances. For example, a claim would likely have been obvious if design needs or market pressures existing in the prior art made it natural for one or more known components to be combined, where each component continues to function in the expected manner when combined (i.e., when there are no unpredictable results). A claim is also likely invalid as having been obvious where it is the combination of a known base system with a known technique that can be applied to the base system without an unpredictable result. A claim is further likely to be invalid as having been obvious if it is a combination of familiar elements according to known methods when it does no more than yield predictable results. Additionally, if a technique has been used to improve one method, and a person of ordinary skill in the art would have recognized that it would improve similar methods in the same way, using the technique would have been obvious unless its actual application would have been beyond the skill of an ordinary person in the art.

26. I have been asked by counsel to assume that when considering obviousness, one must not refer to teachings in the specification of the patent itself as prior art. One can, however, refer to portions of the patent specification that acknowledge certain things as prior art, including the “BACKGROUND” section.

V. Technical introduction.

A. The '686 Patent (Ex. 1001).

27. The '686 patent relates to separating hemoglobin (HbA₂) in a blood sample from other hemoglobin variants such as HbA, HbF, HbS, in that same blood sample by capillary electrophoresis (CE) and then quantifying the amount of the HbA₂. The amounts of each of the variants are referred to as fractions.

28. The '686 Patent explains that separating HbA₂ from the other hemoglobin variants in the blood sample by capillary electrophoresis (CE) was known in the prior art. (Ex. 1001, page 1001-0016, col. 1, lines 11-22)

29. The '686 Patent, in general terms, describes the limitations of prior art techniques that were known to the inventors for separating HbA₂ from other variants (such as HbA, HbC, HbE, HbD, HbF, HbS) in terms of low resolution of the fractions and lack of rapidity of migration time.

30. Specifically, the '686 Patent confirms the problems with the prior art as follows:

- a. Free solution haemoglobin separation has been described, but does not satisfy the expected criteria for accuracy, resolution or rapidity to be able to rationalize CE haemoglobin analyses.

- b. Ishioka (Ex. 1006) described the separation of haemoglobins using a borate buffer (100 mM) at a pH of 9.8 with a migration time of the order of 50 minutes, i.e. incompatible with currently expected times for analyzing haemoglobins.
- c. The same type of buffer under similar pH and concentration conditions (Jenkins) (Exs. 1008, 1009, 1010) can only produce insufficient resolutions between the HbA, HbF and HbS fractions.
- d. Sahin (Ex 1007) describes more acidic pH conditions but obtained deeply unimpressive results for resolution between the HbA, HbF, HbS and HbA₂ fractions with lower concentrations (20 mM) of borate or with barbital (50 mM) at a pH of 8.5, and also deeply unimpressive results for resolution between the HbA, HbS and HbA₂ fractions for Tris buffer (1 M) at a pH of 8.0.
- e. Further, Tris/arginine combinations (Shihabi) (Ex. 1011) have been used and even though they allowed HbA/HbS separation, HbC/HbE and HbA₂ fractions were not resolved.

f. Finally, U.S. Pat. No. 5,202,006 (Ex.1016) and U.S. Pat. No. 5,439,825, (Ex. 1017) which describe the use of barbital or ethyl barbital, could only produce low resolutions between the principal haemoglobins, namely HbA, HbF, HbS and HbC.

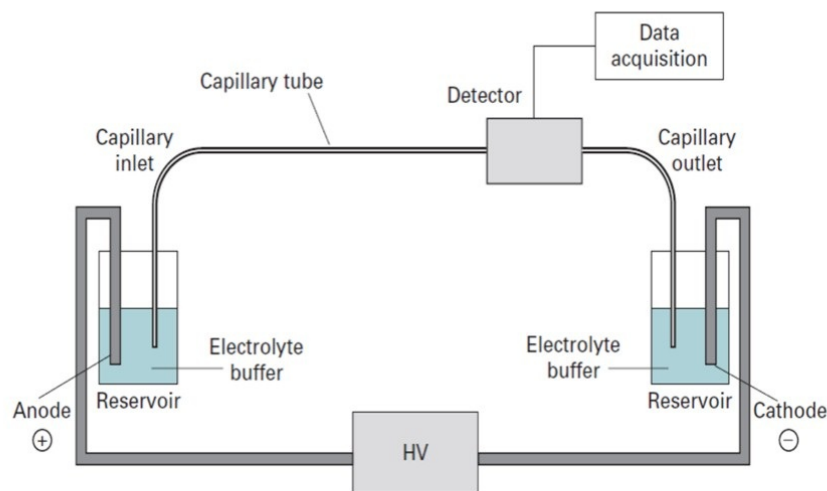
31. Thus, capillary electrophoresis (CE) systems for separating the hemoglobin variants were well-known as of the filing date of the '686 patent.

B. General Technical Concepts.

32. Capillary electrophoresis is taught as part of analytical chemistry to undergraduate chemistry students. There are no drawings in the '686 patent (and no need for such drawings) illustrating capillary electrophoresis.

33. The following is an illustration of a conventional prior art capillary electrophoresis system. The sample to be evaluated is placed in a buffer solution (also referred to as an electrolyte) and a high voltage is applied between the anode (+) and the cathode (-). The voltage causes the sample to flow through the capillary tube (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

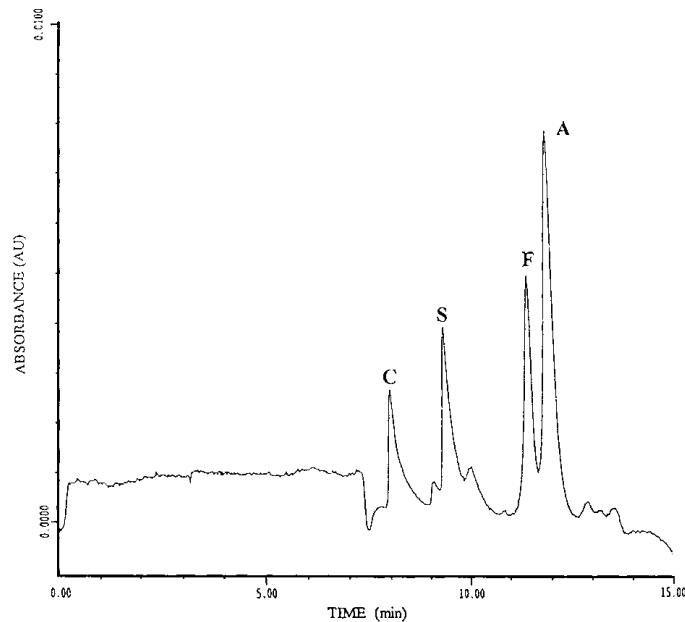
constituents of the sample flow through the tube, they flow past a detector which obtains information, (qualitative and quantitative) and the sample then proceeds to the outlet of the tube. The tube may have a window or clear area such that the detector can respond to the components of the sample.



34. The area identified where the detector is located is referred to as the zone where data is acquired by the system. The terms capillary electrophoresis and capillary zone electrophoresis are used interchangeably.

35. Using human blood as the “sample” being evaluated, the output or result of one type of capillary electrophoresis is illustrated in the following from Ex. 1015 page 1015-0002, using standard prior art CE equipment, with the

separated components of the sample being observed at a wavelength of 214 nm, and with four hemoglobin variants HbA, HbF, HbS and HbC identified.



36. The various peaks in the graph relate to the various components in the sample and the area under the graph between adjacent valleys relate to the amount of each component.

37. Undergraduate students are taught basic concepts of capillary electrophoresis including but not limited to the variables such as magnitude of the voltage, diameter of the tube, pH of the solution (electrolyte), the strength (molarity) of the solution and the constituents or components of the solution.

C. The '686 Patent Claims and the related disclosure.

38. I have been asked by counsel to opine specifically with respect to independent claims 1, 23 and 24, and dependent claims 2-22 of the '686 patent. These claims read as follows:

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-alkylanimonium 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 an 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.

39. Independent claim 1 conceptually has two steps, the first being “introducing a sample... into a capillary tube” and the second being “electrophoresing the sample... where the analysis buffer is of the zwitterionic type and [includes] at least one flow inhibitor.” 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. Independent claim 24 has the same two steps but requires the flow inhibitor to be of the aliphatic or cyclic diamine or polyamine type – details which also appear in claim 4.

40. The ‘686 patent provides examples of “known, commercially available” zwitterionic buffers, referring to them as “Tris” type buffers (Ex. 1001, page 1001-1017, Column 3, line 54 – Column 4, line 7).

41. Significantly, the Tris type zwitterionic buffer was used in two of the prior art systems described in the ‘686 patent, specifically, a Tris buffer in the Sahin system (Ex. 1007) and a Tris/arginine buffer combination in the Shihabi system. (Ex. 1011) Ex. 1001, page 1001-0016, Column 1, lines 59-67).

42. The ‘686 patent provides examples of flow inhibitors including 1,4-diaminobutane. (Ex. 1001, pages 1001-0016 and 1001-0017, Col. 2, lines 40 –

58 and Col. 4, lines 8 – 14, the latter referring to 1,4-diaminobutane by its acronym DAB.)

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

43. The ‘686 patent contained 28 original claims.(Ex.1003, pages 1003-0017 through 1003-0019). The claims were replaced with claims 29 – 55 (Ex. 1003, pages 1003-0203 through 1003-0205) prior to any evaluation by the Examiner. The proceedings were limited to claims 29-52 (Ex. 1003, pages 1003-0242 through 1003-0245).

44. The Examiner rejected the claims (Ex. 1003, pages 1003-0243 through 1003-0256) based on a patent publication to Nouadje et al (U.S. 2002/0162744) (Ex. 1023) relating to the use of a zwitterionic buffer (as to some claims, alone or with other prior art as to certain dependent claims) and combined with the patent to Grover (U.S. Patent 6,083,372) relating to the use of a flow inhibitor such as 1,4-diaminobutane(as to other claims). A response was filed (Ex. 1004, pages 1003-0292 et seq) and a final rejection was sent. (Ex. 1003, pages 1003-0343 et seq.)

45. The argument presented to the Examiner was that the Nouadje document contained a translation error and actually related to serum or plasma but

not for separating blood proteins. (Ex. 1003, pages 1003-0372 et seq, esp. page 1003-0401)

46. The Examiner eventually withdrew all prior rejections and presented a new rejection based on the Sunzeri Patent (U.S. Patent 5,536,382 (Ex. 1018) (Ex.1003, pages 1003-0412 et seq) as prior art for the capillary electrophoresis of hemoglobin A1c separation and detection with a tricine buffer and the use of a quaternary amine for flow reversal. The Examiner also indicated that the Camilleri publication (Capillary Electrophoresis, 1997, pages 370-371) teaches the combination of a zwitterionic buffer (tricine) in a concentration of up to 1 M, and 1, 4-diaminobutane (DAB) as a flow inhibitor with a concentration up to 60mM. (Ex. 1003, page 1003-0416).

47. The response successfully argued that Sunzeri detects an analyte of interest 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, the Sunzeri document 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 flow inhibitor that reduces electroosmotic flow (EOF))(Ex. 1003, pages 1003-0458 et seq.)

48. On October 12, 2010, the Examiner agreed to the grant of the patent.

VI. Level of ordinary skill in the art.

A. Introduction

49. Persons of ordinary skill in the art, seeking a technical degree, such as but not limited to chemistry or biochemistry (or having the equivalent experience), are taught various concepts and how to apply those concepts.

50. A simplistic example is a chemistry or biochemistry undergraduate student's knowledge regarding how to increase or decrease the pH of a solution. A likely starting point in the student's teaching would be to use HCl to decrease the pH (make the pH lower or more acidic) and use NaOH to increase the pH (make the pH higher or more alkaline).

51. The choices in the preceding paragraph are merely a starting point – the student would be taught to evaluate the potential for an undesired reaction and, if there is such a potential, then the student would have been taught to consider other candidates for changing the pH of the solution.

52. The purpose of the preceding three paragraphs is to demonstrate that student are taught concepts, and how to implement those concepts, as part of

their undergraduate training. Graduate training may be specifically directed to more detailed problems.

53. The following are some additional examples directed to capillary electrophoresis technology.

54. A graduate student or a laboratory analyst trained in the ordinary skill in the art would know how to conduct experiments to determine the best conditions to resolve the various clinically relevant hemoglobin forms. That is part of the '686 patent.

55. In contrast, a clinical laboratory scientist would know which of the hemoglobin forms are relevant and justify the need for separate hemoglobin A (wildtype) from hemoglobin A2 (found in a patient with beta-thalassemia), S and C (sickle cell disease and trait), F (fetal form), D and E (rare hemoglobinopathies that causes anemia) for analysis and test reporting purposes. This is more the medical diagnostic part and is neither part of, nor required for, the '686 patent.

56. A person of ordinary skill in the art, with basic knowledge of capillary electrophoresis, would know and understand that parameters such as voltage, buffer composition, buffer pH, buffer concentration, and electroosmotic flow (EOF) could be routinely evaluated for obtaining the desired resolution of the fractions of the sample.

57. Persons of ordinary skill in the art would make a starting selection of the parameters, perform capillary electrophoresis on the sample, and compare the results to other prior art forms of resolution of the fractions of the sample. The starting selection would not be arbitrary in the case of capillary electrophoresis but would be based on a review of relevant literature. One purpose of the activities referred to in this paragraph would be to verify or validate the accuracy and efficacy of the selection of the parameters, *i.e.*, validate the capillary electrophoresis results by comparison of those results to prior art results that did not necessarily involve capillary electrophoresis.

58. While a researcher at the forefront of the technology might test dozens if not hundreds or even thousands of combinations of buffer pH, buffer concentration, buffer selection, etc., that is not the situation for the '686 patent because of the known prior art as I will explain below.

59. Similarly, a researcher might test literally hundreds of combinations of buffer pH, buffer concentration, buffer selection, etc., as part of a graduate thesis or postdoctoral work. That also is not the situation for the '686 patent but is the situation for much of the prior art that I am relying on – prior art that was not expressly “applied to the claims” by the Examiner of the '686 patent.

60. I will elaborate on the above, in the specific context of the claims of the '686 patent, in section VI.D. below.

B. The relevant art.

61. In my opinion, the relevant art would be analytical chemistry or analytical biochemistry, *i.e.*, determining the chemical composition and structure of materials and the quantitative and qualitative evaluation of the amounts of those materials and, more specifically, separation by capillary electrophoresis, including separation of hemoglobin variants by capillary electrophoresis.

C. The level of ordinary skill in the relevant art.

62. In considering the level of ordinary skill, I have considered the levels of education and experience of persons working in the field, the types of problems encountered in the field, and the sophistication of the technology. I understand that a person of ordinary skill in the art is a hypothetical person that is deemed to be aware of the content of all analogous prior art.

63. One of ordinary skill in the art of the '686 Patent, at the relevant time frame, would have (1) a baccalaureate degree in Chemistry or Biochemistry, and (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. I reach this conclusion based on numerous factors. One factor is that the '686 Patent itself cites graduate level articles in 7 professional journals as prior art. (Ex 1001, page 1001-0019, Col. 7, lines 6-19) A second factor is my own experience in my undergraduate and graduate studies. A third factor is my own

experience in teaching others. A fourth factor is my own experience in mentoring or advising other who are seeking advanced degrees. The foregoing is a non-exhaustive list of factors. I would also note that a person of ordinary skill in the art could have many years of experience in the art and less formal education.

64. Persons of ordinary skill in the art would have studied separation techniques including liquid and gas chromatography, column chromatography, electrophoresis (both gel and capillary) and would be familiar with the equipment and its use and limitations. In fact, one of the documents cited in the text of the '686 patent (Jenkins "Evaluation of hemoglobin A₂ quantitation assay and hemoglobin variant screening by capillary electrophoresis." *Journal of Capillary Electrophoresis* (1997) 004:3, pages 137-143") (Ex. 1009) provides a comparison of the results of conventional techniques (cellulose acetate electrophoresis, citrate agar electrophoresis, and cellulose chromatography) with the results of capillary electrophoresis. (Ex. 1009, page 1009-0093, left column, under the heading "Conventional Hb electrophoresis techniques" and under the heading "Results" (continuing to the right hand column).

65. Persons of ordinary skill in the art would be experienced in applying scientific approaches, principles and techniques to problem solving. Thus, for example, a person of ordinary skill in the art may have taken an

analytical biochemistry college course and specifically learned how to separate proteins of biological interest.

D. Practical application of the level of ordinary skill in the art.

66. The '686 patent describes 9 possible buffers: TPAS, ATABS, AMPSO, bicine, HERBS, Tris, AMPD, bis Tris propane (with an amino acid) and explains that tricine is preferred. (Ex. 1001, page 1001-1017, Col. 3, line 54 through Col. 4, line 14). The '686 patent describes an even greater number of flow inhibitors (Ex. 1001, page 1001-0016, Col. 2, lines 40-53) concluding with a narrow preferred list of five (Ex. 1001, page 1001-0017, lines 8-12). The '686 patent further identifies 7 pH modifiers (Ex. 1001, page 1001-0017, Col. 4, lines 34-41). This results in $9 \times 5 \times 7 = 315$ experiments without even taking into account the various examples within the range of pH and buffer concentrations listed in the patent. I have no personal knowledge of how the list of possible buffers, possible flow inhibitors, possible pH modifiers, ranges of pH and ranges of buffer concentrations were derived and/or if thousands of different experiments were performed.

67. However, to a person of ordinary skill in the art, hundreds or thousands of experiments were not necessary.

68. The person of ordinary skill in the art would certainly evaluate the prior art before embarking on experimental research.

69. The '686 patent describes the "greatly improved separation" of the hemoglobin variants by capillary electrophoresis based on the addition of flow inhibitor to a zwitterionic buffer. (Ex. 1001, page 1001-0016, Col. 2, lines 11-16)

70. Consider first the zwitterionic buffer. Tris is identified in the '686 patent as being a prior art buffer for capillary electrophoresis for detection of hemoglobin variants. (Ex. 1001, page 1001-0016, Col. 1, lines 65 et seq.) This eliminates the need to test all the other zwitterionic buffers if the "improvement" was the addition of the flow inhibitor.

71. Consider next the buffer concentration range. A range of 0.5mM to 75 mM is described in Shihabi (Ex. 1011, identified in the '686 patent) thus reducing the need to test buffer concentrations outside that range.

72. Consider next the pH. Again, the Shihabi document (Ex. 1011) suggests a pH adjusted to 8.5 reducing the need to test other buffer pH.

73. Consider next the flow inhibitor. Coufal (Ex. 1013) teaches the combination of Tris with ethylenediamine. Ethylenediamine is a known amine. Coufal also teaches the combination of Tris and SDS. SDS functions as a flow inhibitor at a concentration of 50mM thus reducing the need to test other flow inhibitors and other flow inhibitor concentrations. Camilleri discloses both DAB and 1,5-diaminopentane (DAP) as flow inhibitors (both are listed in the '686 patent) at concentrations up to 60mM and distinguishes them from CTAB which is

a flow reverser. (Ex. 1020, page 1020-0002 last paragraph and page 1020-0003, second paragraph) Landers discloses the use of DAB as a flow inhibitor at a concentration of 0.250 mM.

74. pH adjustment: A person of ordinary skill in the art does not require pre-existing publications to understand how this is best accomplished. pH titrations using acids (*e.g.*, hydrochloric) and bases (*e.g.*, sodium hydroxide) are fundamental concepts that are taught to all undergraduates taking a first-year chemistry or biochemistry class. Students taking chemistry or biochemistry laboratory courses specifically conduct buffer adjustments using acid-base titrations.

75. Since a person of ordinary skill in the art is taught the scientific principles and how to apply them, it does not matter if the prior art was related to capillary electrophoresis *per se*, or capillary electrophoresis related to proteins in general (hemoglobin is a protein), or capillary electrophoresis related to hemoglobin. The person of ordinary skill in the art is trained to consider the prior art and determine its applicability to the problem presented.

76. As will be pointed out below, capillary electrophoresis prior art taught everything that is claimed in the '686 patent.

VII. Relevant time frame for determining obviousness.

77. I have been asked by counsel to assume that prior art and obviousness must be evaluated as of at the time of the invention. I have further been asked by counsel to initially consider the time of the invention as being the foreign application priority date of November 18, 2004.

VIII. Claims considered.

78. I have considered all the claims in formulating my opinion.

IX. Claim construction.

79. I have been informed by counsel that I am to interpret the claims as they would be interpreted by one of ordinary skill in the art as of the relevant time frame.

80. 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).

81. To person of ordinary skill in the art, 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.

82. The fact that the flow inhibitor is not specific to the components being separated is also important in defining the relevant art. The relevant art is not limited to hemoglobin separation by capillary electrophoresis, rather, the relevant art is capillary electrophoresis *per se*.

X. Overview of the prior art relied upon for this opinion.

A. Prior art applied in an Office Action.

83. Nouadje, U.S. Patent Publication 2002/0162744 (Ex 1023), now U.S. Patent No. 7,297,244 (Ex. 1024): As I explained above, the Examiner rejected some of the claims (Ex. 1003, pages 1003-0243 through 1003-0256) based on this patent publication as relating to the use of a zwitterionic buffer. The argument presented to the Examiner was that the Nouadje document contained a translation error and actually related to serum or plasma and was not for separating hemoglobin blood proteins. (Ex. 1003, pages 1003-0372 et seq, esp. page 1003-0401).

84. The Nouadje document includes bis-Tris, tricine, TABS, AMPD, AMPSO, and HEPBS (Ex. 1024, page 1024-0006, Col. 4, lines 5-40) as zwitterionic buffers for capillary electrophoresis, all of which are listed in the '686 patent. The Nouadje document provides a preferred pH range of 8-12, preferably 9-11 and more preferably about 10 (Ex. 1024, page 1024-0006, Col. 4, lines 41-46) which differs only slightly from the range in the '686 Patent of 8 to 11, preferably 8-10. The examples in Nouadje were carried out at a pH of 10 (Ex. 1024, page 1024-0007, Col. 5, lines 35-45) and those in the '686 patent were carried out at a pH of 9.19, 9.37 and 9.40. The Nouadje document describes pH modifiers such as lithium hydroxide, sodium hydroxide, potassium, hydroxide, rubidium hydroxide, caesium hydroxide, francium hydroxide, a mono-di, tri- or tetra-alkylammonium

hydroxide containing 1 to 8 carbon atoms in the alkyl portion (Ex. 1024, page 1024-0006, Col. 4, lines 46-53) which is identical to the list of pH modifiers in the '686 patent. The examples in the Nouadje patent of buffer concentration (10 to 500 mM, preferably 20 to 200mM (Ex.1024 page 1024-0006, Col. 4, lines 54-57) which corresponds substantially to the ranges of 20 to 500mM preferably 100 to 250 mM in the '686 patent.

85. The information in the preceding two paragraphs reinforces my explanation that persons of ordinary skill in the art are taught to apply scientific principles; in this case the knowledge of buffers, pH ranges, pH modification, buffer concentration, etc., from one application of capillary electrophoresis (non-hemoglobin – Nouadje) is used in another application of capillary electrophoresis – hemoglobin.

86. Nouadje was used as prior art (Ex. 1003, page 1003-0249) but Nouadje does not teach separation of hemoglobin variants by capillary electrophoresis using a zwitterionic buffer.

87. Alter (Ex. 1022) was cited as prior art for the proposition that whole blood, serum, plasma, cerebrospinal fluid and urine can all be separated and detected using electrophoresis. (Ex. 1003, page 1003-0348).

88. Mohammad (Ex. 1019) was cited as prior art for the proposition that it was known to use hemolyzed blood in capillary electrophoresis for

identification of hemoglobin variants such as C, S, F and A. (Ex. 1003, page 1003-0350).

89. Chen (Ex. 1016) was cited as prior art for capillary electrophoresis of hemoglobin A, A2, F which comprise normal adult blood, and hemoglobin S. (Ex. 1003, page 1003-0254).

90. Sunzeri (Ex. 1018) was cited as prior art (Ex. 1003 page 1003-0415) for the capillary electrophoresis of hemoglobin A1c separation and detection with a tricine buffer and the use of a quaternary amine for flow reversal. However, as pointed out above, Sebia explained that Sunzeri detects an analyte of interest 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, the Sunzeri document detects a single, specific hemoglobin variant rather than separating and resolving multiple hemoglobin variants).

91. Camilleri, *Capillary Electrophoresis* (1997) (Ex. 1020), was applied in an Office Action as prior art evidencing that CTAB can be added to a buffer for capillary electrophoresis in order to reverse the charge on the silica capillary surface, thus creating a positively charged surface. (Ex. 1003, page 1003-0415) and that DAB (1, 4-diaminobutane) was a flow inhibitor. (Ex. 1003, page 1003-0418).

92. Hempe (Ex. 1012) was applied in an Office Action as prior art for the proposition that hemoglobin A1C has a pI point of 6.935 (Ex. 1003, page 1003-0417).

B. Cited prior art not applied in any Office Action.

93. Sahin (Ex 1007) was cited in the patent application but not applied by the Examiner. Sahin taught separating the hemoglobin variants of human blood samples by capillary electrophoresis (referred to as capillary zone electrophoresis, or CZE) and sought to “establish optimum conditions for Hb variant analysis using CZE with uncoated capillaries.(Ex. 1007, page 2, left hand column, lines 5-8). Sahin teaches the use of human blood samples and hemolyzing the samples. (Ex. 1007, page 2, left-hand column, second paragraph after the section heading “2. Experimental.”)

94. Sahin further teaches the importance of the buffer solution vis-à-vis migration of the components and further teaches solutions that are a mixture of two components, as well as the need for pH adjustment in the range of 8.0 to 9.5. Tris buffers are expressly mentioned. (Ex. 1007, page two, right hand column, last paragraph). Various buffer concentrations (molarities) of different buffers from 0.5 to 75 mM are described. (Ex. 1007, page 3, first paragraph).

95. Shihabi (Ex. 1011) was cited in the patent application but not applied by the Examiner. Shihabi teaches, *inter alia*, hemoglobin separation by capillary electrophoresis using a solution including a zwitterionic analysis buffer of two components (Tris and arginine). Shihabi teaches that a buffer of Tris did not provide as good a separation of the HbF variant as a combined arginine-Tris buffer (Ex. 1011, page two, left column, first paragraph under the heading Results and discussion) Shihabi further teaches the effect of changes in the electrophoresis voltage, the sample size, the migration time, and the capillary tube diameter (Id., right hand column). A longer migration time occurs when the flow rate is reduced. Shihabi teaches separation of hemoglobin variants by capillary electrophoresis using a zwitterionic buffer.

C. Newly Cited Prior Art.

96. Huang (Ex. 1015) teaches capillary electrophoresis separation of hemoglobin variants using a zwitterionic buffer, modifying or adjusting the pH (referred to in a dependent claim of the '686 patent); Tris and HEPES as alternative zwitterionic buffers (referred to in narrower dependent claims of the '686 patent), and that better separation of the hemoglobin variants occurs with a buffer additive. It should be remembered that capillary zone electrophoresis and capillary electrophoresis refer to the same equipment and same technique. Huang expressly refers to using a capillary electrophoresis instrument. (Ex. 1015, page 2,

right hand column, Section 2.2 CZE.) Huang teaches that by increasing pH to 6.65, hemoglobin variants F and A (HbF and HbA) were well separated. (Ex. 1015, page 3 and Fig. 1). This prior art document was not cited by Sebia or the Examiner.

97. Coufal (Ex. 1013) teaches basic principles of capillary electrophoresis separation, the importance of buffer selection, the use of zwitterionic buffer and a flow inhibitor, 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 zwitterionic buffer (referred to in narrower dependent claims of the '686 patent); a concentration of zwitterionic buffer within the range of a narrower dependent claim of the '686 patent; two different flow inhibitors, one of which falls within the narrower dependent claims of the '686 patent, and that better capillary electrophoresis separation occurs with the use of a buffer additive. This prior art document was not cited by Sebia or the Examiner.

98. Coufal expressly uses a combined zwitterionic buffer (TRIS) and a flow inhibitor (SDS) for multiple tests (Ex. 1013, page 9, Table 10, Set 9 Column S6 and Set 5, Column S9). Coufal confirms that some additives (*e.g.*, SDS) are used to modify the EOF and that SDS results in an approximate 10% drop in electroosmotic mobility (depending upon the buffer composition).(Ex. 1013, page 9, left-hand column, Section 3.6) Indisputably, Coufal teaches the

solution of a combination of a zwitterionic buffer component (TRIS) and an EOF reducer (SDS). Coufal also teaches a solution with the combination of a zwitterionic buffer (tris) and a flow inhibitor (ethylenediamine). (Ex. 1013, page 1, summary) Ethylenediamine is an aliphatic diamine. The language “aliphatic diamine” appears in dependent claims of the ‘686 patent.

99. Coufal also teaches the person of ordinary skill in the art that the pH may be in the range of 6.8 to 9.5 (Ex. 1013, page 2, Table 1) and that the zwitterionic buffer concentration may be 20 mM and the flow inhibitor concentration may be 50mM (Id.)

100. The prior art illustrates that the purportedly novel and non-obvious feature, the use of a flow inhibitor in the capillary electrophoresis 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.

XI. Claim-by-claim Obviousness Analysis.

A. Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23.

101. **Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23.** These claims 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).

102. 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 flow inhibitor buffer additive. Coufal teaches various zwitterionic buffer/flow inhibitor combinations and flow inhibitor buffer additives to reduce EOF and thus increase the separation of the components of the sample.

103. It would have been obvious to a POSITA to modify Shihabi or Huang by adding the flow inhibitor of Coufal. Shihabi and Huang both relate to capillary electrophoresis separation of hemoglobin variants and the need for modification of EOF for improved separation of the components and Coufal teaches basic principles of capillary electrophoresis separation. Shihabi and Huang each teach a hemoglobin variant separation by capillary electrophoresis, identical to the claims of the '686 Patent except for the flow inhibitor to enhance separation, a feature that is expressly taught by Coufal for capillary electrophoresis.

104. Because adding the flow inhibitor 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 in this part XI.A. would have been obvious to a POSITA prior to the invention date, all as explained in the following claim charts.

105. Claim Chart for Claim 1.

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
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:	Shihabi teaches everything in the preamble of the claim.	Huang teaches everything in the preamble of the claim.
introducing said biological sample into	Shihabi teaches everything in the first step of the	Huang teaches everything in the first step of the

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
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	method including use of an arginine-Tris buffer (Tris is a zwitterionic buffer), but Shihabi does not teach the use of the flow inhibitor. Coufal teaches use, in capillary columns, of a solution including a zwitterionic buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that another flow inhibitor (SDS) provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013,	method including the use of a zwitterionic buffer (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 flow inhibitor. Coufal teaches use, in capillary columns, of a solution including a zwitterionic buffer (Tris) and including at least one

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
	page 0001 (Summary) and page 0009).	flow inhibitor (ethylenediamine) and that another flow inhibitor (SDS) provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013-page 0001 (Summary) and 1013 page 0009).
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	Shihabi identifies the separated hemoglobin variants A, F, S, and C and A ₂ (Ex. 1011 page 0001) and that separation was better with a Tris buffer having an additive (arginine) as compared to a	Huang teaches the step of electrophoresing and that the hemoglobin variants are A, F, S and C (Ex. 1015 page 0002, Figure 1 and Table 1).

Claim 1	Shihabi in view of Coufal	Huang in view of Coufal
haemoglobin HbA2 and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin.	plain Tris buffer (Ex. 1001 page 0002).	

106. Claim Chart for Claim 2.

Claim 2	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, further comprising	See the claim chart for claim 1.	See the claim chart for claim 1.
detecting said haemoglobins.	Shihabi detects the separated hemoglobin variants A, F, S, and C and A ₂ (Ex 1011 page 0002, Fig 1)	Huang detects the hemoglobins A, F, S and C (Ex. 1015 page 0002, Figure 1 and Table 1).

107. Claim Chat for Claim 3

Claim 3	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
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.	Shihabi teaches use of a Tris buffer. (Ex.1011-page 0001). Coufal teaches use of a tris buffer. (Ex. 1013 page 0001). The '686 patent confirms that a tris 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 page 0017, Col. 3, lines 54-64).	Huang teaches use of a bis-tris propane zwitterionic buffer. (Ex. 1015, page 0001). Coufal teaches use of a tris buffer. (Ex. 1013 page 0001). 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

Claim 3	Shihabi in view of Coufal	Huang in view of Coufal
		opposite to the acid function. (Ex. 1001 page 0017, Col. 3, lines 54-64).

108. Claim Chart for Claim 6.

Claim 6	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
said analysis buffer has a concentration in said analysis buffer solution from about 20 mM to about 500 mM.	The zwitterionic buffer concentration in Coufal is 20mM (Ex. 1013,Table 1). Capillary electrophoresis buffers (non-zwitterionic buffer) within this range (<i>e.g.</i> 100 mM) are confirmed as prior art by the '686 patent. (Ex.1001 page 0016).	The zwitterionic buffer concentration in Coufal is 20mM (Ex. 1013,Table 1). Capillary electrophoresis buffers (non-zwitterionic buffer) within this range (<i>e.g.</i> , 100-mM) are confirmed as prior art by the '686 patent. (Ex.1001 page 0016).

	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.	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.
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109. Claim Chart for Claim 7.

Claim 7	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
said analysis buffer has a concentration in said analysis buffer solution from about 100 mM to about 250 mM	Buffers (non-zwitterionic buffer) within this range are confirmed as prior art by the '686 patent. (Ex.1001 page 0016). The range in the claim would have been obvious	Buffers (non-zwitterionic buffer) within this range are confirmed as prior art by the '686 patent. (Ex.1001 page 0016). 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.	to a POSITA as there does not appear to be any criticality of the range and/or its end points.
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110. Claim Chart for Claim 10.

Claim 10	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
said capillary tube is naked.	Shihabi teaches tubes as being washed and rinsed.(Ex.1011 page 0001). Coufal describes the tubes as bare silica. (Ex.1013 page 0001). The '686 patent confirms that naked tubes are prior art. (Ex. 1001-0016 (col. 1, lines 36-38)	Coufal teaches bare silica tubes. (Ex.1013 page 0001). The '686 patent confirms that naked tubes are prior art. (Ex. 1001 page 0016 (col. 1, lines 36-38) and page1018 (col. 5, lines 6-15).

Claim 10	Shihabi in view of Coufal	Huang in view of Coufal
	and page 1018 (col. 5, lines 6-15). The use of a naked capillary tube would have been obvious to a POSITA.	The use of a naked capillary tube would have been obvious to a POSITA

111. Claim Chart for Claim 11.

Claim 11	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
said capillary tube is formed from fused silica.	Coufal teaches that the tube “can also” be coated (Ex. 1013 page0001) The ‘686 patent refers to fused silica capillaries as prior art. (Ex. 1001 page 0002 and page 0018, col. 5, lines 6-15.)	Huang disclose fused silica capillaries. (Ex. 1015 page 0001) Coufal teaches that the tube “can also” be coated (Ex. 1013 page 0001). The ‘686 patent refers to fused silica capillaries as prior art. (Ex. 1001-0002;

Claim 11	Shihabi in view of Coufal	Huang in view of Coufal
	The use of a fused silica capillary tube would have been obvious to a POSITA	Ex.1001-0018, col. 5, lines 6-15.) The use of a fused silica capillary tube would have been obvious to a POSITA

112. Claim Chart for Claim 12.

Claim 12	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
said analysis buffer solution has a pH from about 8 to about 10.	Shihabi teaches a pH of 8.5 (Ex. 1011page 0001). Coufal teaches analysis buffers with a pH from 6.8 to 9.5 (Ex. 1013 page 1008). It would have been obvious to a POSITA to	Huang teaches prior art analysis buffers with pH of 8.6 (among others)(Ex.1015 page 0001). Coufal teaches analysis buffers with a pH from 6.8 to 9.5 (Ex. 1013 page 0008).

Claim 12	Shihabi in view of Coufal	Huang in view of Coufal
	use a pH range of 8 to 10 for the analysis buffer.	It would have been obvious to a POSITA to use a pH range of 8 to 10 for the analysis buffer.

113. Claim Chart for Claim 13.

Claim 13	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.
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 page 0001) The '686 patent confirms that capillary electrophoresis is used in the prior art to detect abnormal hemoglobins. (Ex. 1001 page 0002).	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 capillary electrophoresis on the sample.

Claim 13	Shihabi in view of Coufal	Huang in view of Coufal
	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 capillary electrophoresis on the sample. It also would have been obvious to a POSITA that the blood sample could be one of the types that are enumerated in the claim.	The ‘686 patent confirms that capillary electrophoresis is used in the prior art to detect abnormal hemoglobins. (Ex. 1001 page 0002). 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 capillary electrophoresis on the sample. It also would have been obvious to a POSITA that the blood sample could be one of the

Claim 13	Shihabi in view of Coufal	Huang in view of Coufal
		types that are enumerated in the claim.

114. Claim Chart for Claim 14.

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

115. Claim Chart for Claim 17.

Claim 17	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 1, wherein	See the claim chart for claim 1.	See the claim chart for claim 1.

Claim 17	Shihabi in view of Coufal	Huang in view of Coufal
said analysis buffer solution further comprises a pH modifier.	Shihabi teaches adjusting the pH. (Ex. 1011 page 0001). Coufal teaches adjusting the pH of the Tris buffer (Ex. 1013 page 0002, Table 1). It would have been obvious to a POSITA that the pH of the buffer solution can be modified or adjusted.	Huang teaches adjusting the pH of the running buffer (Ex. 1015 page 0002). Coufal teaches adjusting the pH of the Tris buffer (Ex. 1013 page 0002, Table 1). It would have been obvious to a POSITA that the pH of the buffer solution can be modified or adjusted.

116. Claim Chart for Claim 18.

117. Number not used.

Claim 18	Shihabi in view of Coufal	Huang in view of Coufal
The method according to claim 17, wherein	See the claim chart for claim 17.	See the claim chart for claim 17.

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 page 0002). It would have been obvious to a POSITA that sodium hydroxide (NaOH) is used to adjust or modify the pH of a solution.	Coufal teaches the use of sodium hydroxide. (Ex 1013 page 0002). It would have been obvious to a POSITA that sodium hydroxide (NaOH) is used to modify the pH of a solution.

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

118. Claim Chart for Claim 23.

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
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	Shihabi teaches everything in the preamble of the claim including hemolysis of the sample. (Ex.1011 page 0001). The '686 patent confirms that capillary electrophoresis is used in the prior art to detect abnormal hemoglobins. (Ex. 1001-0002)	Huang teaches everything in the preamble of the claim except the specific nature of the sample. 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

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
consisting of normal blood, abnormal blood, washed blood, decanted blood, centrifuged blood, whole blood and haemolyzed blood, said method comprising:	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 capillary electrophoresis on the sample. It also would have been obvious to a POSITA that the blood sample could be one of the types that are enumerated in the claim.	capillary electrophoresis on the sample. The ‘686 patent confirms that capillary electrophoresis is used in the prior art to detect abnormal hemoglobins. (Ex. 1001page 0002) 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 capillary electrophoresis on the sample. It also would have been obvious

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
		to a POSITA that the blood sample could be one of the types that are enumerated in the claim.
introducing said biological sample into a capillary tube comprising a solution of an analysis buffer, wherein said analysis buffer is of the zwitterionic buffer type and at least one flow inhibitor, and	Shihabi teaches everything in the first step of the method including use of an arginine-Tris buffer (Tris is a zwitterionic buffer), but Shihabi does not teach the use of the flow inhibitor. Coufal teaches use, in capillary columns, of a solution including a zwitterionic buffer (Tris) and including at least one flow inhibitor	Huang teaches everything in the first step of the method including the use of a zwitterionic buffer (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 page 0001) but does teach the use of the flow inhibitor.

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
	(ethylenediamine) and that another flow inhibitor (SDS) provided a 10% EOF reduction and that other buffer -flow inhibitor combinations provided a 20% reduction. (Ex. 1013 page 0001 (Summary) and page 0009).	Coufal teaches use, in capillary columns, of a solution including a zwitterionic buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that another flow inhibitor (SDS) provided a 10% EOF reduction and other buffer – flow inhibitor combinations provided a 20% reduction. (Ex. 1013 page 0001 (Summary) and page 0009).
electrophoresing said biological sample in	Shihabi teaches the step of capillary electrophoresis	Huang teaches that the step of electrophoresing

Claim 23	Shihabi in view of Coufal	Huang in view of Coufal
said analysis buffer and at least one said flow inhibitor, whereby said haemoglobins are separated by migration.	resulting in separation by migration (Ex.1011 page 0003). Coufal teaches the use of both a zwitterionic buffer and flow inhibitor and refers to the migration time (Ex. 1013 page 0002). It would have been obvious to a POSITA that capillary electrophoresis causes components of the sample to be separated by migration.	involves migration of the hemoglobin variants. (Ex. 1015 page 0001). Coufal teaches the use of both a zwitterionic buffer and flow inhibitor and refers to the migration time. (Ex. 1013 page 0002) It would have been obvious to a POSITA that capillary electrophoresis causes components of the sample to be separated by migration.

B. Claims 15 and 16.

119. **Claims 15 and 16.** These claims 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).

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

121. Accordingly, the claims in this part XI. B. would have been obvious to a POSITA prior to the invention date as explained in the following claim charts.

122. Claim Chart for Claim 15.

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 Claim Chart for Claim 1.	See the Claim Chart for Claim 1.
said analysis buffer is selected from tricine , TAPS, TABS, AMPSO, bicine or HEPBS, or an association of Tris, AMPD or bis Tris	Camilleri teaches selecting the zwitterionic buffer from tricine among other zwitterionic buffers for capillary electrophoresis. (Ex.1020 page 0002).	Camilleri teaches selecting the zwitterionic buffer from tricine among other zwitterionic buffers for capillary electrophoresis. (Ex.1020 page 0002).

Claim 15	Shihabi in view of Coufal and Camilleri	Huang in view of Coufal and Camilleri
propane with an amino acid.		

123. Claim Chart for Claim 16.

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 Claim chart for Claim 15.	See the Claim chart for Claim 15.
said analysis buffer is tricine .	Camilleri teaches selecting the zwitterionic buffer from tricine among other zwitterionic buffers for capillary electrophoresis. (Ex.1020 page 0002)	Camilleri teaches selecting the zwitterionic buffer from tricine among other zwitterionic buffers for capillary electrophoresis. (Ex.1020 page 0002)

C. Claims 4, 5, 8, 9, 19-22 and 24.

124. 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). These claims address details of the flow inhibitor commonly used in capillary electrophoresis but not disclosed in Shihabi or Huang or Coufal. Rather, they are disclosed in prior art documents such as Landers.

125. Accordingly, the claims in this part XI. C. would have been obvious to a POSITA prior to the invention date as explained in the following claim charts.

126. Claim Chart for Claim 4.

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 claim chart for claim 1.	See the claim chart for claim 1.
said at least one flow inhibitor is of an aliphatic or a cyclic diamine or a polyamine type.	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Ethylenediamine is an aliphatic diamine.	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Ethylenediamine is an aliphatic diamine.

Claim 4	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017)	Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017)scope of the claim.

127. Claim Chart for Claim 5.

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 claim chart for claim 1.	See the claim chart for claim 1.
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 which is a zwitterionic buffer and ethylenediamine, an aliphatic diamine, which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB).	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine, an aliphatic diamine, which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB).

Claim 5	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017)	The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017)

128. Claim Chart for Claim 8.

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 claim chart for claim 1.	See the claim chart for claim 1.
said at least one flow inhibitor has a concentration from about 0.01 mM to about 50 mM in said	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001).	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001).

analysis buffer solution.	Ethylenediamine is an aliphatic diamine. Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017) Landers teaches the DAB concentration of 250 μM (Ex. 1026 page0003) which is 0.250 mM within the range of the claim.	Ethylenediamine is an aliphatic diamine. Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017) Landers teaches the DAB concentration of 250 μM (Ex. 1026 page0003) which is 0.250 mM within the range of the claim.
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	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.	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.
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129. Claim Chart for Claim 9.

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 claim chart for claim 1.	See the claim chart for claim 1.
said at least one flow inhibitor has a concentration from about 0.10 mM to about 20 mM in said analysis buffer solution.	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001).	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001).

	Ethylenediamine is an aliphatic diamine. Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017) Landers teaches the DAB concentration of 250 μM (Ex. 1026 page0003) which is 0.250 mM within the range of the claim.	Ethylenediamine is an aliphatic diamine. Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017) Landers teaches the DAB concentration of 250 μM (Ex. 1026 page0003) which is 0.250 mM within the range of the claim.
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	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.	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.
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130. Claim Chart for Claim 19.

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 claim chart for claim 1.	See the claim chart for claim 1.
said an (sic. “at”) least one flow inhibitor is selected from the group consisting of 1,3-diaminopropane, 1,4-diaminobutane, 1, 5-diaminopentane, 1,6-	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001).

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.	putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026- page 0001) and confirms that putrecine is 1,4- diaminobutane (Ex. 1026 page0002) (DAB).	Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026- page 0001) and confirms that putrecine is 1,4- diaminobutane (Ex. 1026 page0002) (DAB).
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131. Claim Chart for Claim 20.

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 claim chart for claim 19.	See the claim chart for claim 19.
said at least one flow inhibitor is selected	Coufal teaches the combination of Tris which	Coufal teaches the combination of Tris which

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.	is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026-page 0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026 page0002) (DAB).	is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026-page 0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026 page0002) (DAB).
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132. Claim Chart for Claim 21.

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 claim chart for claim 20.	See the claim chart for claim 20.

said at least one FI is 1,4-diaminobutane.	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026-page 0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026 page0002) (DAB).	Coufal teaches the combination of Tris which is a zwitterionic buffer and ethylenediamine which is a flow inhibitor to reduce EOF(Ex. 1013 page 0001). Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026-page 0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026 page0002) (DAB).
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133. Claim Chart for Claim 22.

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.	See the immediately preceding chart for claim 21.
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). 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). It would have been obvious to a POSITA to use chemicals in various forms such as salt and/or hydrochloride.	Coufal teaches the combination of Tris (ZB) and ethylenediamine (FI) to reduce EOF(Ex. 1013-0001). 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). It would have been obvious to a POSITA to use chemicals in various forms such as salt and/or hydrochloride

134. Claim Chart for Claim 24.

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
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	Shihabi teaches everything in the preamble of the claim.	Huang teaches everything in the preamble of the claim.
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 zwitterionic buffer

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
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	a zwitterionic buffer), but Shihabi does not teach the use of the flow inhibitor. Coufal teaches use, in capillary columns, of a solution including a zwitterionic buffer (Tris) and including at least one flow inhibitor (ethylenediamine) and that another flow inhibitor (SDS) provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013	(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 page 0001) but does teach the use of the FI. Coufal teaches use, in capillary columns, of a solution including a zwitterionic buffer (Tris) and including at least one flow inhibitor

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	page 0001 (Summary) and page 0009). Ethylenediamine is an aliphatic diamine. Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026 page 0001) and confirms that putrecine is 1,4-diaminobutane (Ex. 1026 page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017)	(ethylenediamine) and that another flow inhibitor (SDS) provided a 10% EOF reduction and other buffer/flow inhibitor combinations provided a 20% reduction. (Ex. 1013 page 0001 (Summary) and page 0009). Ethylenediamine is an aliphatic diamine. Landers teaches use of putrecine as the flow inhibitor in capillary electrophoresis (Ex.1026-0001) and confirms that putrecine is 1,4-

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
		diaminobutane (Ex. 1026-page 0002) (DAB). The '686 patent confirms that DAB is an "aliphatic or a cyclic diamine or a polyamine" (Ex. 1001 page 0017)
electrophoresing said biological sample in said analysis buffer and at least one said flow inhibitor, whereby said haemoglobins are separated by migration.	Shihabi teaches the step of capillary electrophoresis resulting in separation by migration (Ex.1011 page 0003). Coufal teaches the use of both a zwitterionic buffer and a flow inhibitor and refers to the migration time (Ex. 1013 page 0002)	Huang teaches that electrophoresing involves migration of the hemoglobin variants. (Ex. 1015 page 0001). Coufal teaches the use of both a zwitterionic buffer and a flow inhibitor and refers to the migration time. (Ex. 1013-0002)

Claim 24	Shihabi in view of Coufal and Landers	Huang in view of Coufal and Landers.
	It would have been obvious to a POSITA that capillary electrophoresis causes components of the sample to be separated by migration.	It would have been obvious to a POSITA that capillary electrophoresis causes components of the sample to be separated by migration.

Verification and signature.

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

Executed this 2 day of April, 2024.


 Alan H.B. Wu, Ph.D.