

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HELENA LABORATORIES CORPORATION.,
Petitioner,

v.

SEBIA,
Patent Owner.

Case IPR2024-00801
Patent 7,887,686

**DECLARATION OF JAMES P. LANDERS, Ph.D.
IN SUPPORT OF PATENT OWNER'S PRELIMINARY RESPONSE**

TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	MY BACKGROUND AND QUALIFICATIONS	2
III.	FREE SOLUTION CAPILLARY ELECTROPHORESIS OF HEMOGLOBINS	8
IV.	THE PROBLEM IN THE ART OF RESOLVING HEMOGLOBINS	13
	A. Camilleri	13
	B. Chen U.S. 5,202,006	13
	C. Sahin	16
	D. Shihabi	19
V.	OVERVIEW OF THE ‘686 PATENT	21
VI.	COMPARISON OF THE ART RELIED ON BY PETITIONER WITH THE ART IDENTIFIED DURING PROSECUTION	29
	A. Shihabi	30
	B. Coufal	33
	C. Huang	37
	D. Camilleri	38
	E. Landers	39
VII.	PETITIONER’S GROUNDS OF INVALIDITY	40
	A. Preliminary Considerations	40
	1. Resolution	40
	2. What Petitioner Asserts is Missing from Prior Art Applied to the Claims	41
	3. Flow Inhibitor	43
	4. Relevant Art	44
	5. Person of Ordinary Skill in the Art	47
	B. Detailed Analysis of U.S. 7,887,686	50
	1. Separating and Resolving HbA2 and at Least One Other Form of Haemoglobin Samples	52

2.	Separating and Resolving in a Blood Sample.....	54
3.	Analysis Buffer of the Zwitterionic Type and At Least One Flow Inhibitor.....	56
C.	Ground 1: Shihabi or Huang and Coufal Fail to Teach All of the Claim Limitations of Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23 Requiring Capillary Electrophoresis Using a Blood Sample	59
D.	Ground 1: Shihabi or Huang and Coufal Fail to Teach All of the Claim Limitations of Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, and 18 Requiring Resolution of HbA ₂ and HbC, HbD, HbE, HbS, HbF or HbA.....	60
E.	Ground 1: Petitioner Fails to Demonstrate a Motivation to Combine Shihabi or Huang with Coufal with Any Reasonable Expectation of Success.....	63
F.	Ground 2: Claims 15 and 16 Depend from Claim 1 and Camilleri Does Not Provide Teachings Lacking in Shihabi or Huang and Coufal.....	69
G.	Ground 3: Claims 4, 5, 8, 9 and 19-22 Depend from Claim 1 and Landers Does Not Provide Teachings Lacking in Shihabi or Huang and Coufal. Shihabi or Huang and Coufal and Landers Does Not Render Claim 24 Obvious.....	69
APPENDIX A		A-1

I, James P. Landers, do hereby declare as follows.

I. INTRODUCTION

1. I am over the age of eighteen and competent to make this declaration.
2. I have been retained as an expert witness on behalf of Sebia for the above-captioned *inter partes* review (“IPR”). I am being compensated for my time in connection with this IPR at my standard consulting rate, which is \$550.00 per hour.
3. I understand that this Declaration accompanies Patent Owner’s Preliminary Response to a Petition for *Inter Partes* Review filed by Helena Laboratories Corporation. The Petition involves claims 1-24 of U.S. Patent No. 7,887,686 (“the ‘686 patent”) (Ex. 1001). I also understand that the ‘686 patent is currently assigned to Sebia. The ‘686 patent relates to the separation and resolution of hemoglobin (alternatively identified as “haemoglobin”, “Hb”, or “Hgb”) variants in a blood sample using a method commonly referred to as “free solution capillary electrophoresis,” or “capillary zone electrophresis” (CZE). The claimed method uses the combination of a zwitterionic analysis buffer and a flow inhibitor to achieve separation of the hemoglobin variants as indicated by peaks and “guarantee[s] satisfactory resolution between the HbA₂ and HbC, HbD, HbE, HbS, HbF or HbA” variants of hemoglobin (if they are present in the sample) so as to “improve the accuracy and precision of qualitative and quantitative analysis of the variants.” Ex.

1001 at col. 3, lines 2-3. This improved separation and resolution is shown in the qualitative (e.g., distinct separate peaks) and quantitative (calculation of % composition) analysis of the variants present in the samples of Figs. 1-5, 6A-D and 7A-D. Separation and resolution of peaks for qualitative and quantitative analysis requires that the peaks are sufficiently resolved or separated, have a Gaussian shape without excessive peak broadening and a clean baseline. If these conditions are met, one can use commercial software and machines to determine the peak area and a percentage amount of the hemoglobin variant with confidence, as the '686 patent demonstrates.

4. I understand that the '686 patent (Ex. 1001) resulted from U.S. Patent Application No. 11/280,025 ("the 025 application"), filed November 16, 2005. The '025 application is based on a French application FR 04 12263 filed November 18, 2004. I understand that the '025 application claims the benefit of the filing date of the French application FR 04 12263.

II. MY BACKGROUND AND QUALIFICATIONS

5. I am an expert in the field of analytical methods and technologies used to separate and identify biomolecules; this includes the use of CZE to separate and resolve biochemical proteins, such as hemoglobins. I have been actively working and researching in the capillary electrophoresis field since 1990. My primary area of expertise is the analysis of proteins, including the separation of bioactive

compounds from a variety of body fluid samples (blood, urine, saliva, semen, vaginal fluid, etc.) biochemical variants using a variety of methods, e.g., gel and capillary electrophoresis, chromatography, mass spec, acoustic trapping, exploiting differences of size, structure, polarity and charge.

6. In 1983, I received a Bachelor of Science in Biochemistry with a Biomedicine minor from the University of Guelph, Ontario, Canada. In 1988, I received a Doctor of Philosophy in Biochemistry from the Department of Chemistry and Biochemistry, University of Guelph, Ontario, Canada. My Ph.D. involved characterizing receptor proteins using gel electrophoresis.

7. From 1988 to 1989, I was a research fellow at the Banting Institute, University of Toronto Medical School, Toronto, Canada, where my research focus was endocrinology.

8. From 1990 to 1994, I was a research fellow at the Canadian Medical Research Council (MRC) at the Mayo Clinic, Rochester, MN, where I procured the 3rd capillary electrophoresis (CE) system off the assembly line of Beckman Instruments, and I explored the power of CE as a separations technique. My research was primarily focused on CE, and led to the publication of thirteen CE papers between '91-'94; including Landers et al., "High-Performance Capillary Electrophoresis of Glycoproteins: The Use of Modifiers of Electroosmotic Flow for Analysis of Microheterogeneity", *Analytical Biochemistry* 205, 115-124 (1992)

which is Ex. 1026 relied on by Petitioner. In 1993, I edited the 1st edition of the *CRC Handbook on Capillary Electrophoresis*.

9. In 1994, I founded and directed the Clinical Capillary Electrophoresis Facility, within the Division of Clinical Biochemistry and Immunology, in the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN. In support of this new Clinical Capillary Electrophoresis Facility, Beckman Instruments provided ten (10) CE instruments and funded two technicians with the research goal of exploring ‘unmet analytical needs’ in Laboratory Medicine. This involved developing novel CE conditions for resolving analytes, like proteins, and often with no literature precedence. We addressed everything from rapid serum protein profile generation and urine-based kidney function assays, through to drug abuse detection and identification of serum monoclonal proteins. Relevant publications are listed in my CV as references 53, 58, 62, 66, and 73.

10. From 1996 to 1997, I was appointed as Associate Consultant (equivalent of Assistant Professor) in the Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN where I began to establish my own research group. It was here where I edited the 2nd edition of the *CRC Handbook on Capillary Electrophoresis*.

11. In 1997, I joined the Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, as an Assistant Professor, where I began my academic

career building a laboratory with Ph.D. students and post-doctoral fellows, while teaching the undergraduate Analytic Chemistry course and the graduate-level Separations course.

12. In 1999, I moved to the University of Virginia where I was an untenured Associate Professor in the Department of Chemistry with co-appointment as an Associate Professor of Pathology at the University of Virginia Health Sciences Center, Charlottesville, VA. Up to this point, the major focus of my research was ‘Method Development’ for capillary electrophoresis with a diverse array of analytes ranging from ions (e.g., bromide ion), small molecule drugs and biologics, to peptide/proteins and nucleic acids.

13. In December 2001, I was promoted to the rank of Full Professor with tenure in the Department of Chemistry, University of Virginia. From 2001-2006, I published seven (7) papers describing CE methods for protein or peptide analysis, while my group expanded into other areas of interest where CE could be exploited. In 2006, I edited the 3rd and final edition of the *CRC Handbook on Capillary Electrophoresis* (still used today) expanding it to incorporate CE-compatible *Microtechniques*. In 2008, as a result of work incorporating electrophoresis into integrated microfluidic systems, I was honored with the ‘*Innovative Technology of the Year*’ Award from the *Association for Laboratory Automation*. Coincidentally,

I became the microfluidics/separations *Editor* for the journal *Analytica Chimica Acta*.

14. In December 2015, as a result of publishing more than 125 papers since joining the University of Virginia, I became a Commonwealth Endowed Professor.

15. In 2021, I was appointed *Editor-in-Chief* of *Analytica Chimica Acta*, which occupies the rank of 8th out of 100 analytical journals. To-date, I have authored or co-authored over 290 published, peer-reviewed articles, the vast majority focusing on some form of separation technique, with some of these publications involving separation of proteins.

16. I have authored or co-authored 28 text book chapters, many of which relate to capillary electrophoresis, in particular CZE, and clinical diagnostics. I currently co-author two chapters in the “*Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*”, which has long been viewed as the ‘Bible for Laboratory Medicine’. In the 4th - 7th editions of this reference manual, I was the lead author of the chapter on ‘*Electrophoresis*’ and, additionally, in the 5th - 7th editions, I was the lead author of ‘*Microfabrication and Microfluidics and Their Application in Clinical Diagnostics*’.

17. In addition to my educational training and professional and research experiences described above, I have kept abreast of the field of capillary electrophoresis by reading scientific literature, teaching, conferring with colleagues

in the field, attending, presenting and leading scientific conferences and delivering regular and invited lectures.

18. I am also a named inventor on many issued patents and published patent applications.

19. My accomplishments are listed on my CV (Ex. 2002).

20. In particular, I have many years of laboratory experience using capillary electrophoresis to separate and resolve proteins. Early in my career, I designed and carried out capillary electrophoresis experiments myself and directed the experiments of technicians. At the University of Pittsburgh, I also designed and carried out capillary electrophoresis experiments myself and directed the experiments of PhD students and post doctoral fellows. At the University of Virginia, I have directed undergraduate researchers, PhD students, post-doctoral fellows and sabbatical Professors (from other universities) in their experimentation.

21. Importantly, I have actual experience in using CZE to separate hemoglobin variants in a blood sample. While at Mayo Clinic as an MRC Fellow and then as an Associate Consultant, I worked with Dr. Virgil Fairbanks on hemoglobin variant analysis by CZE. I designed and carried out CZE experiments with the express goal of separating hemoglobin variants. Various conditions I experimented with included coated and uncoated capillaries, capillary length, buffer type, additives (organic and aqueous), pH, applied voltage, etc.

22. Accordingly, I am an expert in the use of capillary electrophoresis in general, and CZE to separate and resolve biochemical proteins, such as hemoglobins.

23. In formulating my opinions expressed herein, I have considered the Petition for Inter Partes Review of U.S. Patent No. 7,887,686 and all documents listed therein (Exs. 1001-1026) at pages iv-vi of the Petition. I will refer to those exhibit numbers herein. In formulating my opinions, I have also relied upon my experience, education, and knowledge in the relevant art.

III. FREE SOLUTION CAPILLARY ELECTROPHORESIS OF HEMOGLOBINS

24. Free solution capillary electrophoresis (also known as CZE) is a separation technique that uses an electric potential to cause analytes in a sample (and eventually in the buffer) to “migrate” within a silica capillary. This is referred to as “electrophoretic mobility (μ_{ep})” which simply represents the migration of a charged analyte under the influence of an applied potential, which is primarily determined by the analytes’ net charge and its mass (size). However, applying a potential to a capillary filled with a liquid buffer containing electrolytes produces an additional effect, one that is only induced by an electrical field – this is “electroosmotic flow” (EOF). That mobility or solution movement is represented as “electroosmotic flow” and refers to the bulk movement or flow of the solution itself within the capillary (μ_{ep}); essentially a “pumping of fluid in one direction powered by an electric field.

As a result, the overall mobility of a charge species (μ_{app} ; apparent mobility) in a capillary under the influence of an electric field is simply represented by:

$$\begin{array}{c} \text{Mobility observed} \\ \downarrow \\ \mu_{app} = \mu_{ep} + \mu_{eof} \\ \begin{array}{c} \text{Mobility due to analyte charge (q) and mass (mass)} \\ \downarrow \\ \mu_{ep} \\ \uparrow \\ \mu_{eof} \\ \text{Mobility due to charge on capillary wall} \end{array} \end{array}$$

In other words, the sum of the driving force is from the analyte itself and the bulk flow of the fluid. Accordingly, overall movement of charged analytes within the capillary is a result of both electrophoretic and electroosmotic flow, and it stands to reason that if the charge on the capillary wall decreases dramatically, the mobility of the analyte is primarily driven by charge and mass of the analyte, i.e. electrophoretic mobility.

25. Capillaries used for electrophoresis are comprised of fused silica, a relatively pure form of glass (silicon dioxide; SiO_2). At the appropriate pH, the inner surface of a fused silica capillary in contact with an electrolyte solution is negatively charged due to the presence of silanol groups (SiO^-). When the capillary is uncoated (bare or naked), the negative charge is exposed to the buffer and sample and small, positively-charged ions (e.g., sodium, Na^+) interact with the negative surface. It is this interaction that generates the bulk flow that is EOF. In contrast, if, the inner surface of the fused silica capillary is “coated” (covalently-modified) with a permanent polymeric or other chemical coating, this serves to shield the negative charge on the capillary wall from the buffer components and analytes. As a result of

reducing the surface negative charge, EOF can be dramatically reduced. Both coated and uncoated (bare or naked) capillaries are used in capillary electrophoresis methods depending on the analytes to be separated.

26. Buffers are commonly used in chemistry/biochemistry to avoid drastic changes in pH whether exposed to an acid or a base, and usually involve two forms of the same compound, one that can buffer ‘acidic’ changes and the other ‘basic’ changes. Because Zwitterionic buffer components carry both positive and negative charges, they exhibit properties that are beneficial to capillary electrophoresis, such as stability, low UV absorbance, low conductivity and compatibility with a variety of analytes, including proteins. The ’686 patent describes a Zwitterionic buffer with at least one amine function, at least one acid function (COOH or SO₃H) and at least one OH in opposite position for separation and resolution to the acid function are of particular interest for the separation/resolution of the Hb variants listed in claim 1. Ex. 1001, 7:37-41 (Claim 3). Exemplary Zwitterionic buffers include tricine, TAPS, TABS, AMPSO, bicine or HEPBS - or an an association of Tris, AMPD or bis Tris propane with an amino acid. Ex. 1001, 8:7-10 (Claim 15).

27. A flow inhibitor (also known as a flow modifier) is a chemical that electrostatically binds to the negative charges in the silica on the inner surface. When the flow inhibitor is part of the buffer solution filling the cavity, the effect is “dynamic coating.” A flow inhibitor is often a small organic molecule compound,

and like sodium Na^+ , has a positive charge. This electrostatic interaction (positive charge binds to negative charge) can dramatically reduce the wall charge, thus ‘passivating’ it, and this can result in a decrease in the magnitude of electroosmotic flow. While EOF always exists to some extent (i.e., never zero), it can be reduced to negligible levels. The ‘686 patent uses a flow inhibitor to reduce EOF which lengthens the separation time, and allows the hemoglobin variants, which have very similar electrophoretic mobility, sufficient time to resolve. I provide the table below identifying single amino acid differences between HbA and hemoglobin variants that result in very similar structure and, as a result, very similar electrophoretic mobilities can be expected. Consequently, hemoglobin variants are closely migrating proteins/species.

Hb A: $\alpha 2/\beta 2$ chains
Hb A2: $\alpha 2/\delta 2$ chains
Hb S same as Hb A except glutamic acid in position 6 of the β -chain is replaced by valine (neutral).
Hb C same as Hb A except glutamic acid in position 6 of the β -chain is replaced by lysine (basic).
Hb E same as Hb A except glutamic acid in position 26 of the β -chain is replaced by lysine (basic).
Hb D same as Hb A except glutamic acid in position 121 of the β -chain is replaced by glutamine (neutral).

As shown in the table, HbA, HbS, HbC, HbE and HbD only differ by a single amino acid substitution a hemoglobin protein complex that includes 4 chains, each around 145 amino acids, thus comprised overall of roughly of 600 amino acids. HbC and HbE DO NOT have an amino acid substitution at all, but simply differ by the position of a single lysine in the β -chain; that is in position 6 of HbC but in position 26 in HbE.

28. Capillary electrophoresis can be used to separate proteins without use of a flow inhibitor or flow modifier. While the capillary electrophoresis separation of proteins can be enhanced by flow modifiers, this is not always the case. In general, a reduction in EOF provides longer ‘on-capillary’ time and extends the opportunity for closely migrating species to be resolved. But, a reduction in EOF cannot be used to predict successful separation and resolution of particular closely-migrating proteins within a sample. In particular, before the invention of the ‘686 patent, the art relied on by Petitioner provided no teaching that the differences among closely-migrating hemoglobin variants would be significant enough for EOF modification to allow resolution. Instead, empirical studies are usually required to ascertain whether separation and resolution of a particular protein from other sample components is enhanced by a flow inhibitor.

29. The effect of flow inhibitors on the magnitude of EOF reduction is not predictable, can be affected by a number of parameters (e.g., buffer type and strength) and must be determined empirically. There is no absolute correlation between a type of flow inhibitor and the reduction in EOF, and there is no absolute correlation between reducing EOF and protein separation and resolution. Accordingly, empirical studies are needed for any given protein variants as to whether a flow inhibitor will reduce EOF in a manner to allow sufficient separation and resolution of the protein variants.

IV. THE PROBLEM IN THE ART OF RESOLVING HEMOGLOBINS

30. The '686 patent is directed to *separating and resolving* hemoglobins *in a biological sample* by migration using a capillary electrophoresis method. Prior to the '686 patent, the art of record during prosecution of the '686 patent recognized the problem of separating hemoglobins, but was unsuccessful in obtaining separation and resolution of HbA₂ and also HbC, HbD, HbE, HbS, HbF or HbA when those hemoglobin variants are present in the sample. Dr. Wu agrees with me that there were "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" (Ex. 1004, ¶ 29) and confirms that some of those problems are described in the '686 Patent (Ex. 1004, ¶ 30). Some of these exemplary limitations and/or problems are summarized below.

A. Camilleri

31. Camilleri (Ex. 1020), cited by the Examiner during prosecution of the '686 patent, recognized that "the separation of protein species varying only in subtle structural differences would be difficult, if not impossible, to achieve." Ex. 1020 at 1020-0002.

B. Chen U.S. 5,202,006

32. Chen U.S. 5,202,006 (Ex. 1016), cited by the Examiner during prosecution of the '686 patent, recognized that "hemoglobins present in a normal

adult are Hemoglobin A ($\alpha_2\beta_2$), which comprises about 97% of the total hemoglobin, Hemoglobin A₂ ($\alpha_2\delta_2$) and Hemoglobin F ($\alpha_2\delta_2$), which comprise the remaining 3%.”

Ex. 1016, col. 1 lines 26-30.

The α chains comprise 141 amino acids and the non- α chains comprise 146 amino acids. The exact type, number and precise sequence of these amino acids is characteristic for each type of globin chain; any alteration in the sequences gives rise to an abnormal globin chain, resulting in the production of abnormal hemoglobin. Abnormal hemoglobins are part of a group of inherited disorders collectively referred to as the “hemoglobinopathies.”

Ex. 1016, col. 1 lines 41-49.

33. Chen further teaches that “[w]ith reference to structural defects, approximately 400 structural abnormalities of the globin chain have been described.” Ex. 1016, col. 1 lines 62-64. Chen identifies Hemoglobin S (HbS), Hemoglobin H (HbH) and Hemoglobin F (HbH) as being clinically significant. Ex. 1016, col. 2 line 1-50. Chen, however, teaches that “unequivocal separation of hemoglobin variants, and in particular hemoglobin variants A, F, S and C, has not been demonstrated using open CZE protocols.” Ex. 1016, col. 4 lines 57-60. Chen teaches that “[g]iven to benefits associated with open CZE, and the necessity for screening for hemoglobin variants, it would be advantageous to have an open tube CZE protocol for analyzing hemoglobin variants.” Ex. 1016, col. 4 lines 61-64.

34. Chen's proposed solution is to use a barbituric acid buffer with capillary zone electrophoresis using an untreated capillary column. Ex. 1016, col. 4 line 66 to col. 5 line 15. The result of that proposed solution is reproduced below as Fig. 1.

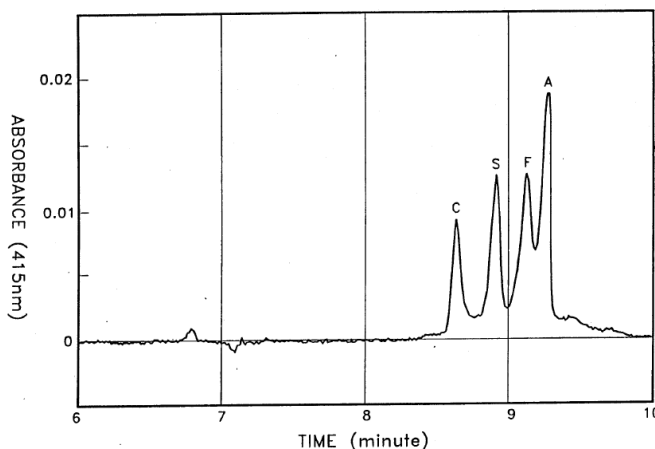


FIG. 1

35. Chen's limitations on separation and resolution were recognized in the '686 patent that Chen "could only produce low resolutions between the principal haemoglobins, namely HbA, HbF, HbS and HbC." Ex. 1001, col. 2 lines 1-4. I have reviewed Chen and Chen does not mention, much less propose a solution for separating and resolving samples containing HbA₂, HbE, or HbD variants and Chen's only solution demonstrates insufficient separation and resolution of HbA, HbF, HbS and HbC variants in a control sample with these hemoglobins. Ex. 1016, Fig. 1.

36. Resolution – how well two peaks are separated – can be defined numerically (as discussed in Huang) by a value that indicates how much 'peak overlap' exists. Peaks that are baseline resolved have a resolution (R) of 1.5, while

peaks that are only partially resolved have an R of less than that. As I described earlier, factors allowing quantitative information to be extracted about two closely-migrating species are: 1) ‘resolution’ (how much overlap), 2) the Gaussian nature of the peaks, 3) how ‘clean’ the baseline is (stability and noise) between peaks. While separation for the C and S peaks of Chen’s Fig. 1 is good, separation between the F and A peaks is low, with poor Gaussian shape and uncertain baseline. Ex. 1016, 1016-0002.

37. I understand that Petitioner agrees with my and the ‘686 Patent’s understanding of the problems/limitations with respect to separation and resolution of a limited number of hemoglobin variants of Chen. See Petition at p. 2 (“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.”)

C. Sahin

38. Sahin (Ex. 1007), of record during prosecution of the ‘686 patent, acknowledges that “hundreds of Hb variants, some producing no symptomatic disease, have been identified by various analytical methods.” Ex. 1007 at 1007-0001. Sahin teaches that “[t]he Hb S and C variants are isoelectric variants as a result of single amino acid changes in one of the globin chains (β), and such changes induce alterations in the structural-functional relationships of the Hb subunits to one

another...” Ex. 1007 at 1007-0001. Sahin proposed using CZE with uncoated capillaries and different buffers to separate hemoglobin variants. Ex. 1007 at 1007-0002.

39. Sahin explains that

[t]he basis for the separation of the Hb variants using CZE is the charge differences in the globin polypeptides. The structure of all variants are such that they possess a net negative charge, under the conditions of CZE separation, and thus the inherent electrophoretic mobility of the Hb species will be directed towards the anode.

Ex. 1007 at 1007-0002. Sahin, however, further discloses that mobility of the Hb variants is ultimately directed towards the cathode because of electro-endosmotic flow (EOF) generated by the electric current and buffer conditions.

However, the use of uncoated fused silica at pH values in the neutral or alkaline range creates a significant electro-endosmotic flow (EOF) directed towards the cathode. The magnitude of the EOF is such that the net mobility of all Hb species is towards the cathode.

Ex. 1007 at 1007-0002. Sahin counsels that “[s]atisfactory separation of analytes using CZE is highly dependent on a suitable electrophoresis buffer-electrolyte” and that “[s]everal different buffers have been used for haemoglobin separations in traditional free zone electrophoresis applications” including Tris. Ex. 1007 at 1007-0002. Sahin further teaches that “[t]he variant Hb F has no significant charge difference from Hb A1 (only 0.05 pH unit separates these variants), and this presents the most difficult challenge to manipulating conditions to separate common variants.” Ex. 1007 at 1007-0003.

40. The '686 patent states that Sahin "obtained deeply unimpressive results for resolution between HbA, HbF, HbS and HbA₂" using a borate or barbital buffer, "and also deeply unimpressive results for resolution between the HbA, HbS and HbA₂ fractions for Tris buffer." Ex. 1001, at col. 1 lines 59-65. I agree and so does Dr. Wu ("the '686 Patent confirms the problems with the prior art" Ex. 1004 at p. 14 para 30.). In general, the figures below show poor Hb variant separation efficiency. More specifically, visual inspection indicates problems with non-Gaussian peak shape (Fig. 4), significant peak overlap (poor resolution) and, an inherent inability to rationally define the baseline for any set of peaks (Figs. 1-3). This is particularly notable with the inability to cleanly resolve HbF from HbA (Fig 3).

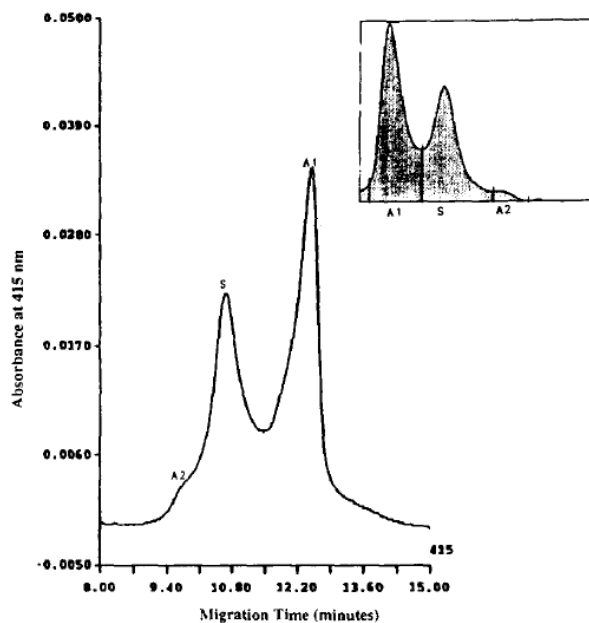


Fig. 1. Electropherogram of a sample from a patient with sickle-cell disease. Electrolyte, 1.0 M Tris-HCl (pH 8.0). Other conditions as described under Experimental.

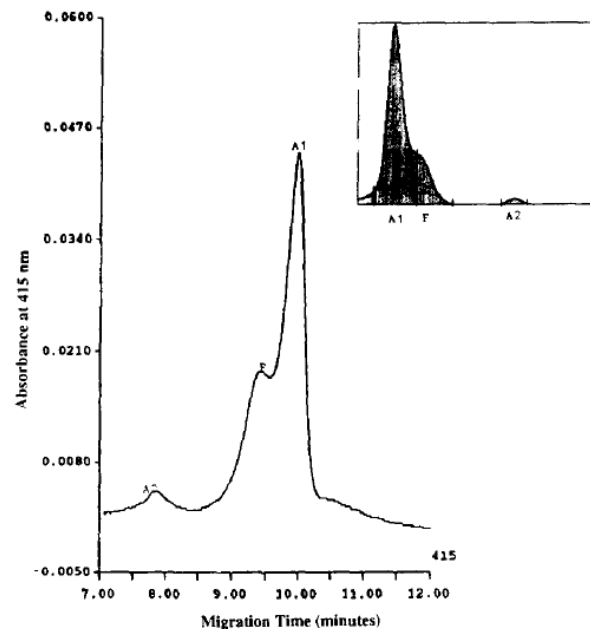


Fig. 2. Electropherogram of a patient's sample with a significant Hb F fraction. Electrolyte, veronal (sodium barbital) (pH 8.5).

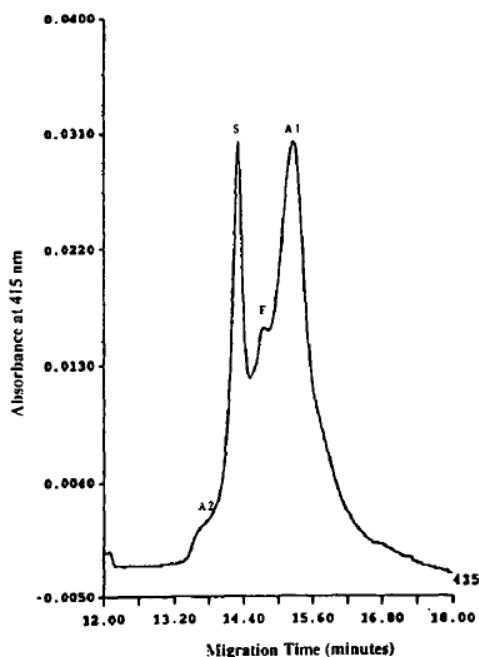


Fig. 3. Electropherogram of a patient's sample containing significant proportions of variants Hb A1, A2, S and F. Buffer, sodium borate (pH 8.5).

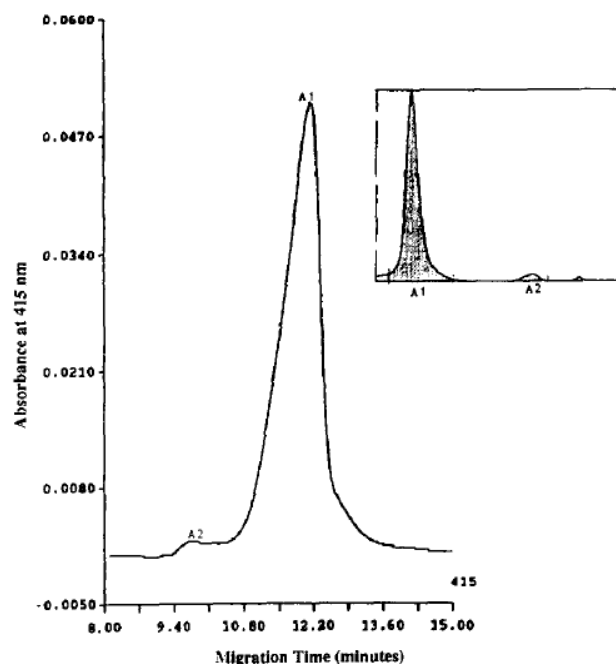


Fig. 4. Electropherogram of a sample obtained from a healthy adult. Buffer, veronal (pH 8.5).

D. Shihabi

41. Shihabi (Ex. 1011), of record during prosecution of the '686 patent, teaches the clinical importance of HbA₂.

An elevated HbA₂ level, in the presence of a normal serum iron level, is used clinically to aid in the diagnosis of β -thalassemia. [Citation omitted]. HbA₂ can also increase slightly in other clinical disorders such as in hyperthyroidism, hemoglobin S (Hb S) trait, and megaloblastic anemia in absence of β -thalassemia.

Ex. 1011 at 1011-0001.

42. Shihabi, however, teaches that separating HbA₂ (being a small peak) is problematic in the presence of a very large peak due to overlap.

However, the two major problems in HbA₂ are the accuracy and the reproducibility of the quantification of a very minor peak (~ 2%) in the

presence of a very large peak. The precision of the HbA₂ should be < 6% for clinical diagnosis. . . . The quantification of a minor small peak in the presence of a large one is problematic in most separation methods and especially CE. In CE, several factors affect the precision and accuracy of such quantification, e.g., sample size, wall interaction, integrator software, and voltage [citation omitted].

Ex. 1011 at 1011-0001.

43. While Shihabi states that “it is demonstrated that the major hemoglobin variants A, F, S, C, and A₂ can be separated well by CE in an arginine-Tris buffer” (Ex. 1011 at 1011-0001), Figure 1 of Shihabi below shows poor Hb variant separation efficiency. More specifically, visual inspection indicates problems with poor separation of A from F, and an inherent inability to rationally define the baseline for quantitation of any of peaks C, S, F and A. See Fig. 1 below using CE at 9 kV.

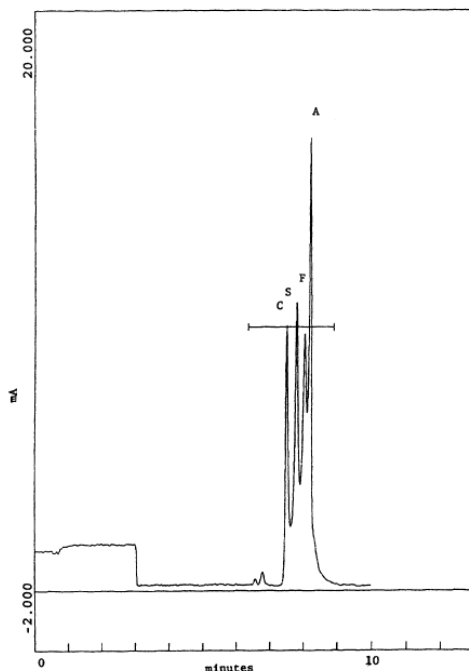


Figure 1. Separation of the major hemoglobin variants by CE at 9 kV.

44. In Shihabi Fig. 1, with the separation voltage of 9 kV, HbA₂ was not identified by Shihabi as a peak. Shihabi reduced the voltage to 6 kV to identify a peak attributable to HbA₂ (Fig. 4). Ex. 1011 at 1011-0002 (“However, for quantification of the HbA₂, the electrophoresis voltage has to be decreased to 6 kV.”). See Ex. 1011, Fig. 4 (bottom).

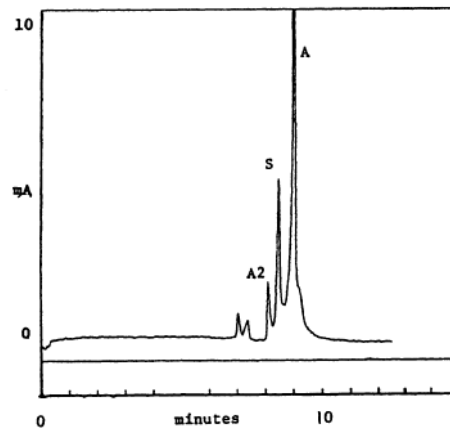


Figure 4. HbA₂ from (top) a normal patient; (middle) a patient with β-thalassemia with a low MCV, and (bottom) a patient with S trait.

45. While a lower applied voltage improved the overall separation of HbA₂, Figure 4 shows poor Hb variant separation efficiency. Visual inspection indicates problems with non-Gaussian peak shape for the A2 and S peaks with fronting and tailing of the A peak especially, and, an inherent inability to rationally define the baseline for any of peaks A2, S and A.

V. OVERVIEW OF THE ‘686 PATENT

46. I have reviewed the ‘686 patent and provide the following analysis.

47. The '686 patent is directed to *separating and resolving* hemoglobins *in a biological sample* by migration using a capillary electrophoresis method. "The term 'haemoglobins' means any normal or abnormal haemoglobin and variants of said haemoglobins." Ex. 1001, col. 1 lines 15-17. The '686 patent states the express goal of separating and resolving hemoglobin HbA₂ from other forms of hemoglobin, namely "a method for analyzing haemoglobin and in particular haemoglobin A₂ which allows single step analysis without a double coating, which can be carried out automatically and in series, and which guarantees satisfactory resolution between HbA₂, HbC, HbD, HbE, HbS, HbF and HbA forms in particular." Ex. 1001, col. 2 lines 5-10. The '686 patent teaches that "the invention can greatly improve the *resolution* of haemoglobin and haemoglobin variants." Ex. 1001, col. 2 line 66 to col. 3 line 1 (emphasis added). The goal of the resolution achieved by the '686 patent is to "improve the accuracy and precision of qualitative and quantitative analysis of the variants compared with analyses carried out using known methods." Ex. 1001, col. 3 lines 1-4. The "qualitative" aspects involve peak identification based on a specific migration time or an observed migration time relative to an internal marker. The "quantitative" aspects involve defining the relative abundance of a specific component(s) based on either 'peak height' or 'peak area'. The latter tends to be more commonly used, where the 'area under the peak' defines how much of that component is present. It is here that a 'stable' baseline with 'low noise' is

important. The '686 patent in particular teaches the separation and resolution of HbA₂ from HbC or HbE, which could not be accomplished by prior art methods. Ex. 1001, col. 3 lines 4-5.

48. The '686 patent details limited success in the field of separating and resolving certain hemoglobins using capillary electrophoresis. According to the '686 patent, the “dynamic double coating” method of Analis kit “cannot, however, separate HbA₂, HbC, and HbE fractions sufficiently well, rendering quantitative analysis of the HbA₂ fraction impossible in the presence of HbC and HbE variants. “Further, since HbS and HbD fractions have the same electrophoretic position, complementary analysis in an acidic medium is necessary to determine the type of variant present in the sample.” Ex. 1001, col. 1 lines 30-46. Ishoka using capillary electrophoresis separated HbC, HbS and HbA using a borate buffer, but provided no indication that its method could separate and resolve other forms of hemoglobin. Ex. 1001, col. 1 lines 52-56. Jenkins (1996), Jenkins (1997) and Jenkins (1999) using capillary electrophoresis could only provide insufficient resolutions between HbA, HbF and HbS fractions. Ex. 1001, col. 1 lines 56-59. Sahin using capillary electrophoresis and a Tris buffer “obtained deeply unimpressive results for resolution between the HbA, HbF, HbS, and HbA₂.” Ex. 1001 at col. 1 lines 59-65. Shihabi using capillary electrophoresis and a Tris-arginine buffer “allowed HbA/HbS separation [but] HbC/HbE and HbA₂ fractions were not resolved. Ex.

1001, col. 1 line 65 to col. 2 line 1. Chen U.S. Patent No. 5,202,006 and Chen U.S. Patent No. 5,439,825 using capillary electrophoresis could only provide “low resolution” for HbA, HbF, HbS, and HbC, and provided no indication that its method could be used to resolve other forms of hemoglobin. Ex. 1001, col. 2 lines 1-4. Accordingly, the prior art identified in the ‘686 patent failed to provide a method to separate and resolve all seven claimed hemoglobin forms when present in a blood sample, especially resolving HbC, HbE and HbA₂ from each other and resolving HbD and HbS from each other.

49. The inventors of the ‘686 patent were the first to discover that the seven forms of hemoglobin, if present in subcombinations in a biological sample, could be separated from other components in the biological sample and resolved into individual peaks by capillary electrophoresis *both qualitatively and quantitatively* using an analysis buffer including *a zwitterionic compound and a flow inhibitor*. Ex. 1001, col. 2 lines 11-26 (“Said step is generally followed by separation of haemoglobins, migration and detection of the various variants.”). See also Ex. 1001, col. 3 lines 6-10 (“The zwitterionic—buffer flow inhibitor associations of the invention are of particular use in analyzing samples in which normal or variant haemoglobins of the HbA₂, HbC, HbD, HbE, HbS, HbF and/or HbA type are present, to detect and/or quantify them.”). The ‘686 patent emphasizes the achievement of

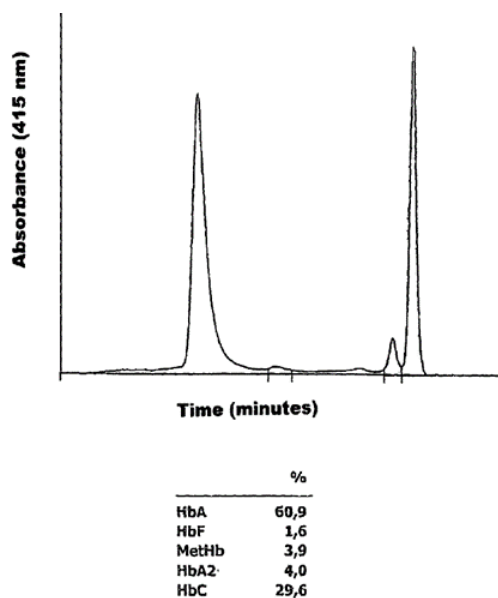
resolving and quantifying HbA₂ even in the presence of HbC and HbE (Ex. 1001, col. 3 lines 4-5). I note that the prior art Shihabi could not achieve this separation.

50. The '686 patent demonstrates the qualitative and quantitative separation and resolution of each of hemoglobins HbA₂, HbC, HbD, HbE, HbS, HbF and/or HbA in different blood samples with different hemoglobin combinations. See Ex. 1001, Figs. 1-7D and col. 3 lines 29-44. The '686 patent provided for the first time a method capable of confirming both the presence and absence of any of the seven forms of hemoglobin, if present, in a blood sample.

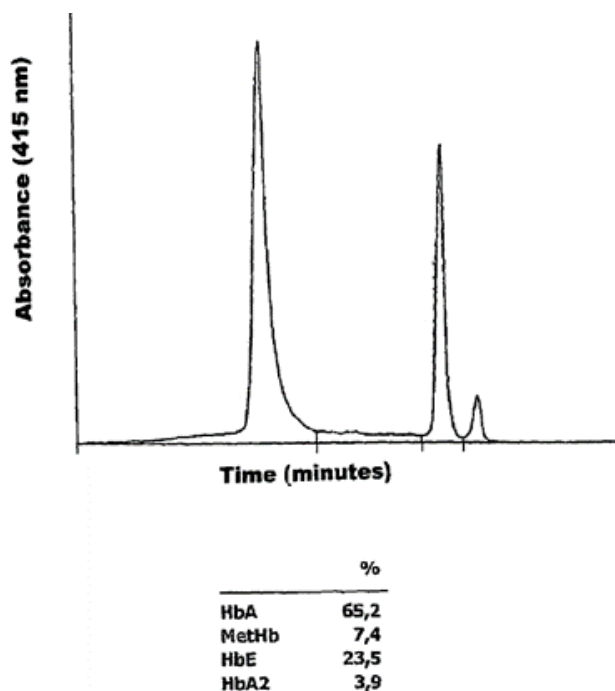
51. Fig. 1 demonstrates the qualitative and quantitative separation and resolution of HbA from HbA₂ in normal human blood, where disease variants are not present.

52. Fig. 2 demonstrates the qualitative and quantitative separation and resolution of HbA, HbF, HbS and HbA₂ in diseased blood where disease variants HbF and HbS are present.

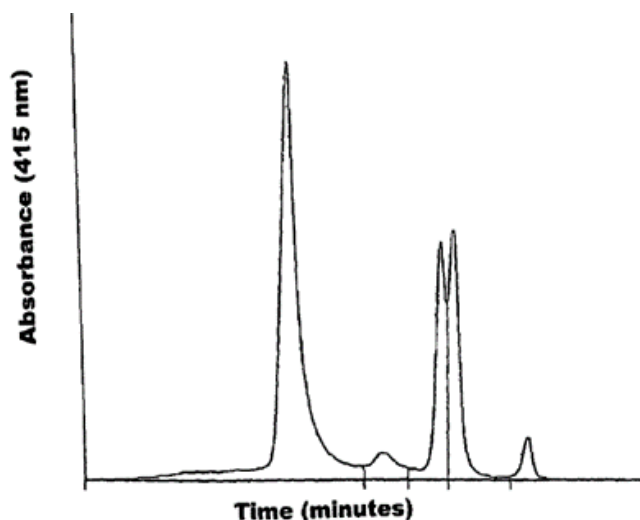
53. Fig. 3 (shown below) demonstrates the qualitative and quantitative separation and resolution of HbA, HbF, metHb, HbA₂ and HbC in diseased blood where variants HbF and HbC are present. Importantly, normal hemoglobin HbA₂ was resolved from variant HbC, which was not achieved by Shihabi or the other prior art references as described in the '686 patent.



54. Fig. 4 (shown below) demonstrates the qualitative and quantitative separation and resolution of HbA, MetHb, HbE and HbA₂ in blood presenting A/E heterozygosity where variant HbE is present. Importantly, normal hemoglobin HbA₂ was resolved from variant HbE, which was not achieved by Shihabi or the other prior art references.



55. Fig. 5 (shown below) demonstrates the qualitative and quantitative separation and resolution of HbA, HbF, HbD, HbS, and HbA₂ in a mixture of blood presenting A/S heterozygosity and blood presenting A/D Los Angeles heterozygosity, where variants HbD and HbS are present. Ex. 1001, col. 6 lines 15-44. Importantly, variant HbD was separated from variant HbS so as to identify the presence of each of HbD and HbS, which was not achieved by Shihabi or the other prior art references as described in the '686 patent.



	%
HbA	55,6
HbF	4,8
HbD	18,4
HbS	18,2
HbA2	3,0

56. For each of the above Figures, the peaks have good separation, good Gaussian shape (no fronting or tailing) and clean baselines, thereby allowing the quantification of the peak area representative of a particular hemoglobin with confidence, as indicated in each Figure. While the peaks in Fig. 5 for D and S are only partially resolved (R is about 0.7), they have good Gaussian shape without fronting, the baseline is clean and a line can be drawn that evenly divides the overlap allowing quantification of the peaks with confidence as has been done in the '686 patent. While the HbA peaks in each exhibit a slight tailing effect, in all cases the HbA is the dominant Hb form and the pre- and post-HbA peak baseline is the same, hence, quantitative value can be extracted.

57. Accordingly, the inventors of the '686 patent successfully demonstrated a method for the separation and resolution of hemoglobin HbA₂, and HbC, HbD, HbE, HbS, HbF or HbA when present in different blood samples by capillary electrophoresis using a buffer including a zwitterionic compound and a flow inhibitor. The '686 patent provides a method not only for detection and quantification of the presence of hemoglobin forms in a blood sample, but also the confirmation of the absence of a particular hemoglobin form, if not detected in the blood sample.

VI. COMPARISON OF THE ART RELIED ON BY PETITIONER WITH THE ART IDENTIFIED DURING PROSECUTION

58. I have been asked to review the art relied on by the Petitioner for alleging invalidity with the art cited (i.e., relied on by the Examiner in rejecting the claims) or of record (i.e., reviewed by the Examiner) during the prosecution history of the '686 patent and to provide my opinion on whether the art relied on by the Petitioner is the same as art cited or of record during prosecution or provides substantially the same teaching as art cited or of record during prosecution. Accordingly, I have reviewed the prosecution history of the '686 patent (Ex. 1003) including the art identified therein and the characterization of the art by the Examiner and the Patentee, as indicated in Appendix A hereto.

59. I have summarized Petitioner’s asserted ground of invalidity as follows.

I note that Huang (Ex. 1015) is relied upon in the alternative to Shihabi (Ex. 1011), with Petitioner making no distinction between the teachings of Shihabi and Huang.

Claims Challenged	35 U.S.C. §	Art Relied Upon
1, 2, 3, 6, 7, 10-14, 17, 18 and 23	103	Shihabi or Huang and Coufal
15 and 16	103	Shihabi or Huang and Coufal and Camilleri
4, 5, 8, 9, 19-22 and 24	103	Shihabi or Huang and Coufal and Landers

A. Shihabi

60. Petitioner relies on Shihabi and Coufal for alleging invalidity of claims 1, 2, 3, 6, 7, 10-14, 17, 18 and 23 (Ground 1). Shihabi was already reviewed and considered by the Examiner during prosecution of the ‘686 patent. Shihabi was listed on an IDS that Applicant submitted to the Office and on December 30, 2008, Examiner Dieterle signed the IDS listing Shihabi, and noted “All references considered except where lined through. /J.D./” (Ex. 1003-0269). No references were lined through and therefore all references were reviewed and considered in determining patentability, including Shihabi. I have been advised that the Examiner signing the IDS is evidence that the Examiner reviewed and considered Shihabi during prosecution of the ‘686 patent.

61. Shihabi is also expressly discussed in the ‘686 patent as failing to teach resolution of “HbC/HbE and HbA₂ fractions” (Ex. 1001, col. 1 line 67) and provides no teachings not already considered by the Office during prosecution of the ‘686 patent. Shihabi teaches separation of hemoglobin variants HbC, HbS, HbF, and HbA using a 9 kV potential and a zwitterionic buffer (Tris). Ex. 1011, Figure 1. Shihabi also teaches separation of HbA₂, HbS and HbA using a lower 6 kV potential. Ex. 1011, Figure 4.

62. During prosecution, the Examiner asserted that Chen U.S. 5,202,006 “recognizes the use of capillary electrophoresis of haemoglobin A, A₂ and F which comprise normal adult blood (col. 1, lines 26-30) and haemoglobin S (col. 2, lines 1-5).” Ex. 1003, pp. 1003-0253-1003-0254; see also Appendix A, ¶¶ 5-8. These are inclusive of the same Hb variants HbA₂, HbS and HbA separated by Shihabi using tris at 6 kV. The Examiner was also aware that Chen taught separation of HbA, HbF, HbS, and HbC using CZE and a barbituric acid buffer, by virtue of this teaching of Chen in the ‘686 patent (Ex. 1001, col. 2 lines 1-5). These are the same Hb variants HbC, HbS, HbF, and HbA separated by Shihabi using tris at 9 kV. The Examiner relied on Mohammad for teaching “the use of hemolyzed blood in capillary electrophoresis for identification of hemoglobin variants such as: C, S, F, and A” using an ampholine buffer, albeit using capillary isoelectric focusing. Ex. 1003, p. 1003-0350; see also Appendix A, ¶¶ 14-18. These are the same Hb variants

HbC, HbS, HbF, and HbA separated by Shihabi using tris at 9 kV. Accordingly, separation of each of the hemoglobins identified in Shihabi (HbC, HbS, HbF, and HbA (tris and 9 kV) and HbA₂, HbS and HbA (tris and 6 kV) was already known, albeit using different buffer conditions and/or isoelectric focusing.

63. During prosecution, Sahin (Ex. 1007) was expressly reviewed and considered by the Examiner by virtue of being listed under OTHER PUBLICATIONS on the '686 patent and on an IDS signed by the Examiner (Ex. 1003, p. 1003-0269) and is described in the '686 patent for teaching "deeply unimpressive results for resolution between the HbA, HbS and HbA₂ fractions for Tris buffer". Ex. 1001, col. 1 lines 63-66. The Examiner was well aware beyond Shihabi that the zwitterionic buffer Tris was described as being used in capillary electrophoresis of samples including Hb variants HbA, HbS and HbA₂ and that the same Hb variants separated by Shihabi were disclosed in the prior art as being separated. In my opinion, Shihabi, therefore, is cumulative of and provides no better teaching than Chen and Mohammad, already known and applied by the Examiner during prosecution, and further including Sahin which was reviewed and considered by the Examiner. Petitioner has provided no explanation of why Shihabi is a better prior art reference than Chen, Mohammad, or Sahin. Of course, Shihabi, itself, was reviewed and considered by the Examiner during prosecution of the '686 patent. Shihabi is not a new prior art reference.

B. Coufal

64. Regarding Ground 1, Coufal (Ex. 1013) is relied on by Petitioner for teaching, in general, a combination of a zwitterionic buffer and a flow inhibitor and in particular Tris as a zwitterionic buffer having an amine function, an acid function and a hydroxyl function and ethylenediamine as an aliphatic diamine flow inhibitor. See Petition page 22 (claim 1) and 43 (claim 23). The Examiner relied on Camilleri for teaching a combination of a zwitterionic buffer and a flow inhibitor (1,4-diaminobutane) (Ex. 1003, p. 1003-0418; see also Appendix A, ¶¶ 26-32). 1,4-diaminobutane is an aliphatic diamine just like the ethylenediamine of Coufal. Camilleri, in general, teaches the use of cationic amines, of which the ethylenediamine of Coufal is one. Ex. 1020 at 1020-0002. Petitioner has provided no explanation of how or why Coufal is a better prior art reference than Camilleri with respect to claims 1 and 23 of Ground 1 for the teaching of a zwitterionic buffer and a flow inhibitor in general. In my opinion, Coufal's teachings for the use of a zwitterionic buffer and a flow inhibitor are the same as Camilleri already considered by the Examiner with respect to Ground 1, claims 1 and 23.

65. Petitioner has also relied on Coufal's ethylenediamine for teaching the aliphatic diamine of Ground 3, claims 4, 5, and 24. See Petition page 49 (claim 4), page 51 (claim 5) and 63 (claim 24). However, as I stated earlier, Camilleri teaches 1,4-diaminobutane, which is an aliphatic diamine just like Coufal's

ethylenediamine. Petitioner has provided no explanation of how or why Coufal is a better prior art reference than Camilleri with respect to a teaching of the aliphatic diamine of claims 4, 5 and 24 of Ground 3. In my opinion, Coufal's teachings for the use of a zwitterionic buffer and an aliphatic diamine flow inhibitor are the same as Camilleri already considered by the Examiner with respect to Ground 3, claims 4, 5, and 24.

66. Petitioner has relied on Coufal for teaching a zwitterionic buffer concentration of 20 mM which is within the claimed range of about 20 mM to about 500 mM of claim 6 of Ground 1. See Petition at p. 27. However, the Examiner relied on Sunzeri for teaching a 50 mM tricine buffer. Ex. 1003, p. 1003-0416; see also Appendix A, ¶¶ 26-32. A tricine buffer is a zwitterionic buffer. Petitioner has provided no explanation of how or why Coufal is a better prior art reference than Sunzeri with respect to a teaching of the zwitterionic buffer in a concentration of 50 mM of claim 6 of Ground 1. In my opinion, Coufal's teaching for the use of a zwitterionic buffer concentration of 20 mM is substantially the same as Sunzeri already considered by the Examiner with respect to Ground 1, claim 6.

67. Petitioner has relied on Coufal for teaching bare silica tubes as the naked capillary tube of claim 10. Petition at p. 30. However, Coufal is not a better prior art reference than "the '686 patent [confirming] that naked tubes are prior art." Petition at p. 30-31. Petitioner has provided no explanation of how or why Coufal

is a better prior art reference than Petitioner's assertion based on the '686 patent that "naked tubes are prior art." In my opinion, Coufal's teaching for the use of bare silica tubes is the same as or substantially the same Petitioner's assertion based on the '686 patent that "naked tubes are prior art" with respect to Ground 1, claim 10.

68. Petitioner has relied on Coufal for the teaching of "coated" silica tubes as the capillary tube formed from fused silica of claim 11. Petition at p. 32. However, Coufal is not a better prior art reference than "the '686 patent [referring] to fused silica capillaries as prior art." Petition at p. 32. Petitioner has provided no explanation of how or why Coufal is a better prior art reference than Petitioner's assertion based on the '686 patent representing "fused silica capillaries as prior art." In my opinion, Coufal's teaching for the use of coated silica tubes is the same as or substantially the same Petitioner's assertion based on the '686 patent representing "fused silica capillaries as prior art" with respect to Ground 1, claim 11.

69. Petitioner has relied on Coufal for the teaching of an analysis buffer with a pH of from 6.8 to 9.5 as being within the claimed pH range of from about 8 to about 10 of claim 12. Petition at p. 34. However, Petitioner states that Shihabi teaches a pH of 8.5. Petition at p. 34. Further, the Examiner relied on Sunzeri for teaching a buffer with a pH of about 7.2 which the Examiner stated fell within the claimed pH range of about 8. Ex. 1003, p. 1003-0419; see also Appendix A, ¶¶ 26-32. Petitioner has provided no explanation of how or why Coufal is a better prior

art reference than Shihabi's pH of 8.5 or Sunzeri's pH of 7.2. In my opinion, Coufal's teaching of a pH from 6.8 to 9.5 is the same as or substantially the same as Shihabi's pH of 8.5 or Sunzeri's pH of 7.2 with respect to Ground 1, claim 12.

70. Petitioner has relied on Coufal for the teaching of adjusting the pH of the Tris buffer as being the pH modifier of claim 17. Petition at p. 38. However, Petitioner states that Shihabi teaches adjusting the pH. Petition at p. 38. Further, the Examiner relied on Nouadje for teaching a pH modifying compound. Ex. 1003, p 1003-0251; see also Appendix A, ¶¶ 5-10. Petitioner has provided no explanation of how or why Coufal is a better prior art reference than Shihabi's teaching of adjusting pH or Nouadje's pH modifying compound. In my opinion, Coufal's teaching of adjusting pH is the same as or substantially the same as Shihabi's teaching of adjusting pH or Nouadje's teaching of a pH modifying compound of Ground 1 claim 17.

71. Petitioner has relied on Coufal for teaching the use of sodium hydroxide to modify pH as recited in claim 18. Petition at p. 40. However, the Examiner relied on Nouadje for teaching a sodium hydroxide pH modifying compound. Ex. 1003, p. 1003-0251; see also Appendix A, ¶¶ 5-10. Petitioner has provided no explanation of how or why Coufal is a better prior art reference than Nouadje's teaching to use sodium hydroxide as a pH modifying compound. In my opinion, Coufal's teaching

of using sodium hydroxide is the same as Nouadje's teaching of sodium hydroxide as a pH modifying compound of Ground 1 claim 18.

C. Huang

72. Huang (Ex. 1015) is relied on by Petitioner for the very same teachings as Shihabi to allegedly invalidate certain claims. See Petition at pages 16, 19-20 ("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."). But, even Petitioner admits that Shihabi and Huang fail to teach "the FI to enhance separation, a feature that is expressly taught by Coufal for CE." Petition at p. 20. I do note that Huang teaches "use of a neutral, hydrophilic coated capillary with no electroosmotic flow (EOF)." Ex. 1015 at 1015-0001. Accordingly, Huang itself teaches that a flow inhibitor is not needed or desired with its method since there is no EOF to reduce.

73. The Petitioner identifies no reason how or why Huang is a better teaching than Shihabi. In fact, the Petitioner treats Huang (which separates HbF, HbA, HbS and HbC) as interchangeable with Shihabi (which also separates HbF, HbA, HbS and HbC). In my opinion, Huang's teachings relied on by Petitioner, therefore, are the same as or substantially similar to Shihabi's teachings relied on by Petitioner, which was already considered by the Examiner.

74. In my opinion, Huang, therefore, is cumulative of and provides no better teaching than Chen and Mohammad, already known and applied by the Examiner during prosecution, and further including Sahin which was reviewed and considered by the Examiner. Petitioner has provided no explanation of why Huang is a better prior art reference than Chen, Mohammad, or Sahin.

D. Camilleri

75. Camilleri (Ex. 1020) is relied on by Petitioner only for teaching the zwitterionic buffer tricine with respect to claims 15 and 16. Petition at pages 46-48. Camilleri is relied on by Petitioner for no other teaching. Camilleri was expressly considered by the Examiner during prosecution of the '686 patent for teaching the combination of a zwitterionic buffer and flow inhibitor 1,4-diaminobutane (Ex. 1003, p. 1003-0418; see also Appendix A, ¶¶ 26-32), but was not relied upon for teaching a tricine buffer. Certainly, the Examiner reviewed and understood that Camilleri taught a tricine buffer. Instead, the Examiner relied on Sunzeri for teaching a tricine buffer. Ex. 1003, p. 1003-0415; see also Appendix A, ¶¶ 26-32. Camilleri was already considered by the Examiner during prosecution. Further, in my opinion, Camilleri's teaching of a tricine buffer being relied on by Petitioner is cumulative of Sunzeri's teaching of a tricine buffer already considered by the Examiner.

E. Landers

76. I am the James P. Landers of the Landers (Ex. 1026) prior art reference. Landers is relied on by Petitioner only for teaching 1,4-diaminobutane as the aliphatic diamine of claims 4, 5 and 24 of Ground 3. Petition at 50 (claim 4), 52 (claim 5) and 65 (claim 24). Landers is relied on by Petitioner for no other teaching. However, as I stated earlier, Camilleri teaches 1,4-diaminobutane, which is an aliphatic diamine. Petitioner has provided no explanation of how or why Landers is a better prior art reference than Camilleri with respect to a teaching of the aliphatic diamine of claims 4, 5 and 24 of Ground 3. In my opinion, Landers's teaching of the use of the aliphatic diamine is the same as Camilleri already considered by the Examiner with respect to Ground 3, claims 4, 5, and 24.

77. Landers is also relied on by Petitioner for teaching the 1,4-diaminobutane of claims 19, 20, 21, and 22 of Ground 3. Petition at page 58 (claim 19), 59 (claim 20), 61 (claim 21) and 62 (claim 22). However, as I previously stated, Camilleri teaches 1,4-diaminobutane. Petitioner has provided no explanation of how or why Landers is a better prior art reference than Camilleri with respect to a teaching of 1,4-diaminobutane of claims 19, 20, 21 and 22 of Ground 3. In my opinion, Landers's teachings for the use of the 1,4-diaminobutane are the same as Camilleri already considered by the Examiner with respect to Ground 3, claims 19, 20, 21 and 22.

78. With respect to claims 8 and 9, Petitioner asserts Landers teaches a flow inhibitor (1,4-diamino butane) in a concentration of 250 μ M (0.250 mM) which is within the claimed range of about 0.01 mM to about 50 mM (claim 8) (Petition at page 54) or within the claimed range of about 0.01 mM to about 20 mM (claim 9) (Petition at page 56). However, Camilleri was cited by the Examiner for teaching the use of 1,4-diaminobutane “with concentrations of up to 60 mM” which encompasses the claimed range. Ex. 1003, p. 1003-0418; see also Appendix A, ¶¶ 26-32. Petitioner has provided no explanation of how or why Landers is a better prior art reference than Camilleri with respect to a teaching of 1,4-diaminobutane in an amount within the claimed ranges of claims 8 and 9 of Ground 3. In my opinion, Landers’s teaching of the use of the 1,4-diaminobutane in a concentration of 250 μ M (0.250 mM) is the same as or substantially similar to Camilleri’s range of up to 60 mM already considered by the Examiner with respect to Ground 3, claims 8 and 9.

VII. PETITIONER’S GROUNDS OF INVALIDITY

79. I have been asked to review the Petitioner’s grounds of invalidity of the claims of the ‘686 patent and to provide my opinions in response thereto.

A. Preliminary Considerations

1. Resolution

80. At Petition p. 2, Petitioner defines “low resolution” as “insufficient separation.” I agree, especially with respect to the references discussed in the ‘686 patent such as Chen ‘006, i.e. Chen “could only produce low resolutions between

the principal haemoglobins, namely HbA, HbF, HbS and HbC.” Ex. 1001, col. 2 lines 3-4. As I described earlier, factors contributing to a qualitative and quantitative separation and resolution of two components are: 1) the amount of overlap, 2) a Gaussian peak shape, and 3) a ‘clean’ the baseline (stable, low noise) between peaks. Certainly, “insufficient separation” or any of the above would at least make quantitative analysis of hemoglobins challenging.

2. What Petitioner Asserts is Missing from Prior Art Applied to the Claims

81. Petitioner states at page 3 that “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.”

Petitioner is wrong as I explain below for a)-d).

82. Regarding a) Hemoglobin variant separation by CE alone or with ZB, the Examiner stated during prosecution that “Chen recognizes the use of capillary electrophoresis of haemoglobin A, A2, F which comprise normal adult blood (col. 1, lines 26-30), and haemoglobin S (col. 2, lines 1-5).” Ex. 1003, pp. 1003-0253-1003-0254. See also, Appendix A, ¶¶ 5-10; Ex. 1016, col. 5 lines 33-36 (“Fig. 1 is an electropherogram of a control sample comprising hemoglobin variants A, F, S,

C, using a 240mM barbital buffer, pH 8.6, by open CZE with an untreated capillary column.”);Ex. 1016, col. 6 lines 1-4 (“a buffer comprising barbituric acid, or the derivatives of barbituric acid, can efficiently discriminate hemoglobin variants in an open CZE system.”). Chen, applied by the Examiner, therefore contemplated “hemoglobin variant separation by CE alone or with ZB.”

83. Regarding b) Any CE separation using both ZB and FI, Camilleri states the problem that “the separation of protein species varying only in subtle structural differences would be difficult, if not impossible, to achieve.” Ex. 1020 at 1020-0002. To address this, Camilleri then states that “zwitterions are frequently used in FSCE” (free solution capillary electrophoresis). Ex. 1020 at 1020-0002. And, “[c]ationic amines such as 1,4-diaminobutane (DAB) and 1,5-diaminopentane (DAP) have also been used to suppress protein-capillary surface interactions at concentrations up to 60 mM.” Ex. 1020 at 1020-0002. See also Petition at page 11 (“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).”). Camilleri, therefore, contemplated “any CE separation using both ZB and FI.”

84. Regarding c) Any CE separation recognizing the need to reduce EOF, in my opinion, preventing a protein-capillary surface interaction as described above

would be understood to reduce electroosmotic flow (EOF). Camilleri, therefore, contemplated reducing EOF in a CE separation.

85. Regarding d) Any CE separation using ZB and FI to reduce EOF, Camilleri also states that “[p]rotein adsorption may also be reduced by addition of cationic surfactants such as cetyltrimethylammonium bromide (CTAB) to the buffer. Increasing the concentration *first reduces*, then eliminates, and finally reverses the charge on the capillary surface, thus creating a positively charged surface (the latter condition also causes a reversal of the EOF).” Ex. 1020 at 1020-0003 (Emphasis added). In my opinion, this describes the use of CTAB in a certain amount *to reduce protein adsorption and thereby reduce EOF*. Camilleri, therefore, contemplated “Any CE separation using ZB and FI to reduce EOF.”

3. Flow Inhibitor

86. Petitioner relies on Dr. Wu for a definition of “flow inhibitor” as “a substance which is not specific to the components being separated and which reduces or decreases the EOF.” Petition at pages 14-15. Ex. 1004, ¶¶80-81. I disagree with Dr. Wu.

87. A flow inhibitor is a chemical used in a free solution capillary electrophoresis method that interacts with the capillary to alter the charge on the inner surface wall of the capillary. This interaction affects electroosmotic flow.

88. The Patentee argued that the claimed flow inhibitor was different from the antibody of Sunzeri because it did not bind to the target analyte. Ex. 1003, pp. 1003-0458-1003-0460; see also Appendix A, ¶¶ 33-38. In this context, the claimed flow inhibitor “is not specific for a particular analyte or complex to be separated”, because the claimed flow inhibitor is not an antibody used to bind to an analyte. Because the explanation was based on Sunzeri’s antibody, the language that the “the flow inhibitor is not specific for a particular analyte or complex to be separated” is not definitional.

89. In any event, I do not believe that a definition of flow inhibitor is needed because I disagree with the Petitioner’s positions of invalidity on other grounds as stated later in my Declaration.

4. Relevant Art

90. However, I do strongly disagree with Dr. Wu’s statement that the “[t]he 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*.” Ex. 1004, ¶82.

91. Dr. Wu is wrong to assert that the statement made by the Patentee about the flow inhibitor defines the relevant art. I have been advised that the scope of the

prior art includes art that is reasonably pertinent to the particular problem with which the invention was involved.

92. Dr. Wu admits that “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.” Ex. 1004, ¶27. Dr. Wu admits that the particular problem in the art was in separating hemoglobins using CE. Ex. 1004, ¶¶28-30.

93. Dr. Wu admits that “prior art is from the same field of endeavor or interest as addressed in the ‘686 patent, namely *hemoglobin variant separation by capillary electrophoresis*” or “the prior art is *reasonably pertinent to hemoglobin variant separation by capillary electrophoresis*” Ex. 1004, ¶3 (emphasis added).

94. Dr. Wu ignores that the claims as a whole are directed to free solution capillary electrophoresis for separating and resolving hemoglobins. Dr. Wu also ignores that the primary references Shihabi and Huang he relies on are directed to capillary electrophoresis of hemoglobins.

95. Accordingly, I disagree that “the relevant art is capillary electrophoresis *per se*.” Ex. 1004, ¶82. Since the particular problem with which the invention was involved is the separation of hemoglobins using capillary electrophoresis as

acknowledge by Dr. Wu, the relevant art is the separation of hemoglobins using capillary electrophoresis.

96. Further, Petitioner defines the relevant art as “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.” Petition at page 19. However, this definition is inconsistent with Dr. Wu’s definition of the relevant art. Dr. Wu defines the relevant art as including analytical biochemistry as an alternative to analytical chemistry. Ex. 1004, ¶32.

97. Further, Dr. Wu “more specifically” defines the relevant art as “separation by capillary electrophoresis” as compared to Petitioner who distances capillary electrophoresis as a mere “example.” Compare Ex. 1004, , ¶61 with Petition at page 19. Further, Dr. Wu expressly includes separation of hemoglobins variants by capillary electrophoresis within the definition of “relevant art”, while Petitioner distances by stating that the relevant art is “not limited to separation of hemoglobin variants by CE.” In essence, Petitioner has attempted to reserve the entire field of analytical chemistry as “relevant art” while Dr. Wu has a more limited opinion. I provide the table below comparing Petitioner’s definition of “relevant art” with Dr. Wu’s definition of relevant art.

Petition — Relevant Art	Dr. Wu — 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.”	“[T]he 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.”
--	--

98. In any event, I do not believe that this issue needs to be resolved at this time in order for me to conclude that the claims of the ‘686 patent are not invalid as asserted in Grounds 1, 2 and 3.

5. Person of Ordinary Skill in the Art

99. Petitioner states that a POSITA “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.” Petition at p. 18. Petitioner’s definition of the POSITA differs notably from Dr. Wu’s definition. I have provided the table below comparing Petitioner’s definition pf the POSITA with Dr. Wu’s definition of the POSITA.

Petition — POSITA	Dr. Wu — POSITA
--------------------------	------------------------

“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.”	“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.”
--	---

100. Dr. Wu’s definition alternatively includes a baccalaureate degree in biochemistry. Petitioner makes no explanation of why the art of analytical biochemistry or the baccalaureate degree in biochemistry is excluded by Petitioner’s definitions of a POSITA, especially when the claimed subject matter is directed to the biochemical application of separating hemoglobins within a blood sample. Indeed, it is unclear whether Dr. Wu has any experience in separating hemoglobins using free solution capillary electrophoresis, despite that being the focus of the claims.

101. Furthermore, Dr. Wu states that “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)”. Ex. 1004, ¶29. Dr. Wu recognizes these variants as being clinically significant and that “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.” Dr. Wu concludes that “[t]his is more the medical diagnostic part and is neither part of, nor required for, the ‘686 patent.” Ex. 1004, ¶55. Having reviewed the prosecution history, Dr. Wu, however, is well aware that claim 1 was amended to expressly select the seven forms of hemoglobin he identifies as being clinically significant for separation. Ex. 1003, p. 1003-0396; see also Appendix A, ¶¶ 19-30. Dr. Wu attributes the selection of the seven forms of hemoglobin to a clinical laboratory scientist, yet provides no requirement that the POSITA have any clinical laboratory experience. Dr. Wu provides no explanation for this notable discrepancy between his definition of the POSITA (and that of the Petitioner) other than to say the claim includes a “medical diagnostic part”. What is clear is that Dr. Wu has relied on no actual laboratory experience of his own, clinical or otherwise, in identifying hemoglobin variants and grouping them for analysis using capillary electrophoresis.

102. Petitioner’s and Dr. Wu’s definitions of the POSITA are in agreement that one of skill is required to have “(3) have at least two years of experience in the capillary electrophoresis.” Compare Ex. 1004, ¶63 with Petition at page 18. However, Dr. Wu does not explain how one of skill obtains at least two years of experience. Presumably, this experience may be obtained in post-graduate research or industry. However, Dr. Wu’s basis for alleging this requirement is entirely based

on (1) the work of others cited in the '686 patent, (2) his own undergraduate or graduate education experience, (3) his teaching experience, and (4) mentoring or advising others. Ex. 1004, ¶63. Dr. Wu cites to no *actual and significant* capillary electrophoresis experience of his own on which to base his definition of a POSITA. Again, it is not at all clear whether Dr. Wu, himself, has ever used free solution capillary electrophoresis to separate hemoglobins or whether he has even observed such a method or reviewed experimental data resulting from such a method.

103. As noted above, the Petition fails regardless of the proposed definitions of the relevant art or the POSITA. As such, I reserve the right to provide my understanding of the relevant art and of a POSITA later in this proceeding should it be instituted.

104. In any event, I do not believe that this issue needs to be resolved at this time in order for me to conclude that the claims of the '686 patent are not invalid as asserted in Grounds 1, 2 and 3.

B. Detailed Analysis of U.S. 7,887,686

105. Independent claims 1, 23, and 24 of the '686 patent are presented below.

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.

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 haemolyzed 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.

106. Claims 1, 23 and 24 each require a free solution capillary electrophoresis method for separating and resolving haemoglobins in a blood sample

by migration using an analysis buffer of the zwitterionic type and at least one flow inhibitor.

1. Separating and Resolving HbA₂ and at Least One Other Form of Haemoglobin Samples

107. Claim 1 further requires that said haemoglobins are a haemoglobin HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin. Accordingly, from among the haemoglobins in a blood sample, claim 1 requires separation and resolution of haemoglobin variant HbA₂ and, if present, claim 1 further requires separation and resolution of hemoglobin variants HbC, HbD, HbE, HbS, HbF or HbA.

108. Accordingly, the method resolves each of an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin, when present in the blood sample. For example, if HbA₂ and HbA are present in the blood sample, then each is resolved by the method. If HbA₂, HbA, HbF and HbS are present in the blood sample, then each is resolved by the method. If HbA₂, HbA, HbF, and HbC are present in the method, then each is resolved by the method. If HbA₂, HbA and HbE are present in the blood sample, then each is resolved by the method. If HbA₂, HbA, HbF, HbD and HbS are present in the blood sample, then each is resolved by the method.

109. I note that the '686 patent states the express goal of separating and resolving haemoglobin HbA₂ from other forms of haemoglobin, namely "a method for analyzing haemoglobin and in particular haemoglobin A₂ which allows single

step analysis without a double coating, which can be carried out automatically and in series, and which guarantees satisfactory resolution between the HbA₂, HbC, HbD, HbE, HbS, HbF and HbA forms in particular.” Ex. 1001, col. 2 lines 5-10. The ‘686 patent in particular teaches the separation and resolution of HbA₂ from HbC or HbE. Ex. 1001, col. 3 lines 4-5. See also Ex. 1001, Figs. 1-5 and discussion at col. 6 lines 15-44 which demonstrate separation and resolution of HbA₂ from other forms of haemoglobin in blood samples. In addition, the ‘686 patent identifies many prior art efforts to separate various haemoglobin forms, but none were reported in the ‘686 patent as being successful in separating HbA₂ from other forms of haemoglobin. Ex. 1001, col. 1 line 49 to col. 2 line 4.

110. I also note that in an Amendment and Response dated December 22, 2009 (Ex. 1003, p. 1003-0396; see also Appendix A, ¶¶ 19-30), the patentee amended the main independent method claim 29 to recite separating haemoglobin HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin. The Patentee did not amend what would become issued claims 23 and 24 in a similar manner, instead choosing the recite “haemoglobins” in claims 23 and 24. The Patentee argued that the cited art Nouadje, Alter, Chen and Mohammad failed to teach separation of HbA₂ from other forms of haemoglobin. Ex. 1003, pp. 1003-0402-1003-0407; see also Appendix A, ¶¶ 19-30. In an Amendment and Response dated July 21, 2010 (Ex. 1003, p. 1003-0452), the Patentee further argued that

Sunzeri, Camilleri and Hempe likewise failed to teach separation of HbA₂ from other forms of haemoglobin. Ex. 1003, pp. 1003-0458-1003-0463; see also Appendix A, ¶¶ 33-38.

2. Separating and Resolving in a Blood Sample

111. Claims 1, 23 and 24 each require separating and resolving haemoglobins in a blood sample. I note that the ‘686 patent teaches that the blood sample “may be normal or abnormal, washed, decanted, centrifuged, or whole.” Ex. 1001, col. 4 lines 18-19. All the Figures of the ‘686 patent demonstrate free zone capillary electrophoresis using blood samples. See e.g., Ex. 1001 at FIGS 1-7D, 3:29-44. The ‘686 patent teaches that “the invention can greatly improve the resolution of haemoglobin and haemoglobin variants.” Ex. 1001, col. 2 line 66 to col. 3 line 1. The ‘686 patent teaches “satisfactory resolution between HbA₂, HbC, HbD, HbE, HbS, HbF and HbA forms in particular.” Ex. 1001, col. 2 lines 5-10. The ‘686 patent in particular teaches the separation and resolution of HbA₂ from HbC or HbE, which could not be accomplished by prior art methods. Ex. 1001, col. 3 lines 4-5; See also Figs. 1-7D and discussion at col. 6 lines 15-65 which demonstrate separation and resolution of HbA₂ from other forms of haemoglobin in various blood samples.

112. I also note that in an Amendment and Response dated April 8, 2009, the Patentee amended claim 1 to recite a “free solution capillary electrophoresis”

method of a “blood sample.” Ex. 1003, p. 1003-0296; see also Appendix A, ¶¶ 10-13. In an Amendment and Response dated July 21, 2010, the Patentee argued that Sunzeri taught use of a labeled reagent (“binding pair member”) to bind to an analyte in a hydrolyzed blood sample “and then employing capillary electrophoresis to resolve the bound from unbound reagent. The sample constituents are then quantitated by measuring directly or indirectly, the bound reagent.” The Patentee argued that Sunzeri taught that the reagent bound to the target analyte is resolved based on the “reagent [being] displaced from its normal migration position” as a result of being bound to the target analyte. Ex. 1003, pp. 1003-0458-1003-0459 (emphasis added) ; see also Appendix A, ¶¶ 33-38. In response to the Patentee’s arguments, the Examiner issued a notice of allowance dated October 12, 2010 allowing claims 29-32, 34-51, 56 and 57 after making an Examiner’s amendment to claims 29, 56 and 57 to insert “and resolving” after “separating” thereby distinguishing “separating” from “resolving.” The Examiner issued the following statement of reasons for allowability (emphasis original):

The instant application is allowable over other art because the instant application teaches a free solution capillary electrophoresis method for **separating and resolving haemoglobins** in a biological sample. The method comprises electrophoresing utilizing an analysis buffer that is a zwitterionic type and at least one flow inhibitor. (Emphasis added.)

The closest prior art of record, Sunzeri (US 5,536,382) teaches a method of capillary electrophoresis for separating and detecting haemoglobin in a **lysed blood sample** utilizing a tris tricine buffer. Sunzeri mixes the sample with a labeling reagent and detects the

labeling reagent's displacement from its normal migration position from unbound reagent. Sunzeri teaches the detection of a single specific analyte, not multiple haemoglobins. In Sunzeri, only the resolution of bound from unbound reagent is taught. However, the present invention not only separates haemoglobins, it also provides for the resolving of the different haemoglobin components such as HbA2, HbC, HbD, etc. (Emphasis added.)

Additionally, Nouadje et al. (US 2002/0162744) was cited, however, while Nouadje et al. teach **a method of electrophoresis for separating blood components which includes haemoglobins**, Nouadje et al. **does not specifically teach the resolution of the different haemoglobin constituents**. Rather Nouadje et al. only teach the detection of haemoglobin in the sample, **it does not resolve the different haemoglobin constituents**.

Ex. 1003, pp. 1003-0472-1003-047(Emphasis added); see also Appendix A, ¶

39

3. Analysis Buffer of the Zwitterionic Type and At Least One Flow Inhibitor

113. Claims 1, 23 and 24 each require use of an analysis buffer of the Zwitterionic type and at least one flow inhibitor. I note that the '686 patent teaches that "the invention concerns the use of compounds known for their electrophoretic flow inhibiting activity in free solution CE in association with at least one zwitterionic buffer." Ex. 1001, col. 2 lines 63-65. "The CE methods employing zwitterionic buffer/flow inhibitor associations of the invention are of particular use in analyzing blood and separating haemoglobin and its variants in human samples." Ex. 1001, col. 4 lines 28-32.

114. I also note that in an Amendment and Response dated December 22, 2009, the Patentee argued that the claimed flow inhibitor was different from the compound of Nouadje that interacts with the hydrophobic portion of an analyte to change the charge/weight ratio of the analyte thereby shifting migration of the analyte:.

(1) A seric protein differs from the other seric proteins by their entire structure. A seric protein can be shifted and therefore better separated from other seric proteins by using specific additives which interact with specific seric proteins. Any additive changing the ratio charge/weight can be used. Amongst these additives, due to the specific hydrophobicity of a seric protein, additive having a hydrophobic portion can be used (see Nouadje).

Ex 1003, p. 1003-0402; see also Appendix A, ¶¶ 19-25.

115. The Patentee argued that the claimed flow inhibitor was different because it did not influence the charge/weight ratio of Hb variants in the blood sample.

When an additive is added to a blood sample for improving the separation of Hb variants, the variants remain in the same order when separated. In other words, the transformation of the profile is homothetic. To the contrary, the seric protein peaks in an electrophoregram are shifted under the influence of the additives having an influence on the ratio charge/weight

Ex. 1003, p. 1003-0403 (emphasis original) ; see also Appendix A, ¶¶ 19-25.

116. The Patentee also argued that the claimed flow inhibitor was different from the positively charged quaternary amine CTAB of Sunzeri. Sunzeri taught use

of CTAB to ultimately reverse the negatively charged silica capillary surface to a positively charged capillary surface and thereby reverse the electroosmotic flow.

117. In an office action dated January 29, 2010, the Examiner argued that Sunzeri described the concept of “electroosmotic flow” and that

[A] quaternary amine (i.e. CTAB) can be used for electroosmotic flow reversal (col. 8, lines 13-15, i.e. giving “flow inhibitor” its broadest reasonable interpretation)(see Camilleri, Capillary Electrophoresis, which evidences 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, see page 371).

Ex. 1003, p. 1003-0415; see also Appendix A, ¶¶ 26-32.

118. In an amendment and response dated July 21, 2010, the Patentee distinguished the claimed flow inhibitor from the CTAB compound of Sunzeri based on the claimed flow inhibitor’s interaction with the capillary surface to reduce electroosmotic flow (EOF), as opposed to interaction with the analyte itself.

In contrast, the methods of the instant invention comprise the use of an analysis buffer of the zwitterionic type which comprises at least one flow inhibitor. 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 an homothetic manner, i.e., the variants remain in the same order when separated.

Ex. 1003, p. 1003-0461; see also Appendix A, ¶¶ 33-38.

119. The Patentee explained that the claimed flow inhibitor “is used to decrease the charge density on the capillary surface, and thus reduce the EOF” as

opposed to reversing the electroosmotic flow, as with the CTAB compound of Sunzeri:

Applicants submit that reversal of EOF by CTAB as disclosed in the prior art is not the same as reduction of EOF, and thus the art does not in fact teach or even suggest the use of CTAB as a flow inhibitor such as described in the methods of the present invention. Specifically, Camilleri teaches that one can reduce the protein adsorption on a capillary surface by increasing the concentration of CTAB in the buffer used during capillary electrophoresis. The addition of CTAB causes a reversal of charge on the capillary surface, thus creating a positively charged surface, which causes a reversal of the EOF. As disclosed by Applicants, however, the flow inhibitor is used to decrease the charge density, and thus reduce the EOF – not to reverse EOF. Indeed, Applicants submit that hemoglobin variants have isoelectric points between about 6 and 7.7; thus at alkaline pH, hemoglobins are negatively charged, as is the capillary surface. If the capillary surface is positively charged, which is the case when reversal is caused, no separation would occur because of the interaction between the positively charged capillary surface and the negatively charged proteins.

Ex. 1003, pp. 1003-0461-1003-0462; see also Appendix, A, ¶¶ 33-38.

C. Ground 1: Shihabi or Huang and Coufal Fail to Teach All of the Claim Limitations of Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23 Requiring Capillary Electrophoresis Using a Blood Sample

120. Claim 1 of the ‘686 patent, and accordingly, dependent claims 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, 18 and 23 each require a free solution capillary electrophoresis method using a blood sample. Shihabi, Huang and Coufal each fail to teach or suggest a free solution capillary electrophoresis method *using a blood sample*.

121. Shihabi teaches using red blood cells and not a blood sample which includes components other than red blood cells. Ex. 1011 at 1011-0002 (“For sample preparation red blood cells were collected in EDTA tubes (10 μ L) and vortex-mixed with 400 μ L hemolysis solution.”) Huang teaches using “[m]ixtures of Hb C, S, F, and A, of Hb A and F, and of Hb A and S” and not a blood sample. Ex. 1015 at 1015-0001. Coufal fails to teach or suggest any biological sample.

D. Ground 1: Shihabi or Huang and Coufal Fail to Teach All of the Claim Limitations of Claims 1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, and 18 Requiring Resolution of HbA₂ and HbC, HbD, HbE, HbS, HbF or HbA

122. Claim 1 of the ‘686 patent, and accordingly, dependent claims 2, 3, 6, 7, 10, 11, 12, 13, 14, 17, and 18 each require separating and resolving haemoglobin HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin. Shihabi and Huang fail to teach or suggest separating and resolving haemoglobin HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin. Coufal fails to teach any hemoglobin separation.

123. Shihabi teaches the problem of separating a small peak such as with HbA₂ with larger peaks using capillary electrophoresis. “[T]wo major problems in HbA₂ are the accuracy and the reproducibility of the quantification of a very minor peak (~ 2%) in the presence of a very large peak.”. . The quantification of a minor small peak in the presence of a large one is problematic in most separation methods

and especially CE. In CE, several factors affect the precision and accuracy of such quantification, e.g., sample size, wall interaction, integrator software, and voltage [9].” Ex. 1011 at 1011-0001. Shihabi recognizes that “HbA₂ comigrates with HbC.” As the Petitioner acknowledges, Shihabi teaches use of a Tris buffer but not a flow inhibitor. Petition at page 3. Ex. 1011 at 1011-0001-1011-0002.

124. Shihabi has no discussion or example of separation efficiency with hemoglobin variants HbD and HbE and, further, has no teaching or description of being able to separate and resolve HbA₂ from these variants. Shihabi’s Figure 1 shows poor Hb variant separation efficiency between hemoglobin variants HbC, HbS, HbF and HbA at 9kV using the Tris buffer. Ex. 1011 at 1011-0002 and Fig. 1. Shihabi was not able to separate and resolve HbA₂ from variants HbC, HbS, HbF and HbA at 9kV using the Tris buffer, insofar as no HbA₂ peak is identified.

125. Shihabi recognizes that HbC and HbE comigrate with HbA₂ in its capillary electrophoresis method. Ex. 1011 at 1011-0003 (“Since Hb C behaves similarly to hemoglobin HbA₂ in migration in this method, it can be used for checking the linearity, with some reservations. Note that when HbA₂ is over 10% this high ratio indicates that the hemoglobin is not HbA₂. Hemoglobin E, which is very common in the Far East, comigrated with Hb C and also with HbA₂ in this system, similar to that observed in the agarose electrophoresis.”).

126. In order to identify a peak corresponding to HbA₂, Shihabi teaches to reduce the voltage to 6kV. Ex. 1011 at 1011-0002 (“However, for quantification of HbA₂, the electrophoresis voltage has to be decreased to 6kV.”) Shihabi does not teach or suggest using a flow inhibitor additive with the Tris buffer to separate and resolve HbA₂. However, when the voltage was reduced to 6kV to separate HbA₂, Shihabi does not show peaks for HbC or HbE. Only peaks attributable to HbS and HbA are identified. See Ex. 1011 at 1011-0003 (Fig. 4).

127. Accordingly, at 9kV, Shihabi was unable to separate and resolve HbA₂. At 6 kV, Shihabi was unable to show peaks for HbC or HbE. Accordingly, Shihabi does not teach a method that separates and resolves HbA₂ and other variant haemoglobins present in the blood sample, unlike the claimed method for separating and resolving HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin, when present in a blood sample.

128. Huang (Ex. 1015) has no discussion, or identification, of peaks for HbD or HbE in any model mixture. Huang identifies peaks for HbC, HbS, HbF and HbA (Ex. 1015, 1015-0002(Fig. 1)), identifies peaks for HbF and HbA (Ex. 1015, 1015-0002(Fig. 2a)) and identifies peaks for HbS and HbA (Ex. 1015, 1015-0002(Fig. 2b)) in model mixtures. However, several problems are apparent, including non-Gaussian peak shape (‘fronting’ and ‘tailing’), significant peak overlap (poor resolution) and, an inherent inability to rationally define the baseline for any given

peak. Huang does not teach the claimed method for separating and resolving HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin when present in a blood sample. Coufal fails to teach or suggest resolution of any haemoglobin variant.

E. Ground 1: Petitioner Fails to Demonstrate a Motivation to Combine Shihabi or Huang with Coufal with Any Reasonable Expectation of Success

129. The Petitioner asserts that Shihabi teaches use of Tris as a zwitterionic buffer but admits that Shihabi fails to teach use of a flow inhibitor. Petition at page 16 (“Shihabi teaches CE separation of hemoglobin variants using ZB and a buffer additive (not an FI).”. The Petitioner further asserts that Huang teaches use of a zwitterionic buffer but admits that Huang fails to teach use of a flow inhibitor. Petition at page 16 and 19 (“Shihabi and Huang . . . do not teach a FI buffer additive.”). Instead, the Petitioner relies on Coufal for teaching “various ZB/FI combinations and FI buffer additives to reduce EOF and thus increase the separation of the components of the sample.” Petition at page 19.

130. The Petitioner concludes that “[b]ecause 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.”

131. I disagree.

132. In my opinion, there is no direct correlation between a flow inhibitor and magnitude of effect on EOF. Furthermore, even if a flow inhibitor reduces the EOF in a free solution capillary electrophoresis system analysis of a blood sample, it is not reasonably predictable that HbA₂ will be separated and resolved, and nor is it reasonably predictable that an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin will also be separated and resolved, when present in a blood sample. Hb variants often stem from very small changes, e.g., a single amino acid substitution, having a negligible effect on mass, and often such changes do not change the overall charge. An even more challenging situation surfaces when the only a change between HbA and either HbC or HbE, is the location of an identical amino acid substitution. More specifically, relative to HbA, both variants, HbC and HbE, have a glutamic acid substituted by lysine, however, in the HbC variant this occurs at position 6, while that in HbE occurs at position 26. This is important because separation in free solution capillary electrophoresis is based on differences in charge/mass (q/m) ratio. Hence, Hb variants would be expected to have very similar, if not identical mobilities, and therefore difficult to separate. Dr. Wu has provided no analysis of the electrophoretic mobilities of hemoglobin variants and how they may be affected by a reduction of EOF.

133. Before the invention date of November 18, 2004, it is simply unknown how the hemoglobin variants may react to a reduction in EOF in terms of separation and resolution. Dr. Wu has provided no evidence of the function of a flow inhibitor on hemoglobin separation and resolution before the invention date of November 18, 2004. Dr. Wu has provided no evidence that reducing EOF will increase the separation and resolution of hemoglobins before the invention date of November 18, 2004, especially whether reducing EOF will separate and resolve HbA₂ and also whether HbC, HbD, HbE, HbS, HbF or HbA will be separated and resolved, when present in a blood sample. This would have to be done empirically.

134. Petitioner provides no scientific justification for asserting that adding a flow inhibitor in the systems of Shihabi or Huang will predictably separate and resolve HbA₂ and also an HbC, an HbD, an HbE, an HbS, an HbF or an HbA, when present in a blood sample. As I stated, simply reducing the EOF does not provide a reasonable expectation that HbA₂ will be separated and resolved and also that an HbC, an HbD, an HbE, an HbS, an HbF or an HbA will also be predictably resolved, when present in a blood sample. This is because hemoglobin variants are complex proteins having the same or similar electrophoretic mobility, and there is no basis identified by Dr. Wu or the prior art, for a conclusion that a flow inhibitor reducing EOF will facilitate separation and resolution of hemoglobins that have the same or similar electrophoretic mobility.

135. Shihabi provides no basis for one of skill to conclude that adding a flow inhibitor will result in separation and resolution of HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin when present in a blood sample. The Petitioner provides no analysis of the structure of the claimed haemoglobins from which one of skill could predict that adding a flow inhibitor to reduce electroosmotic flow will result *in a degree of EOF reduction that will be sufficient to resolve* HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin when present in a blood sample. The Petitioner provides no evidence of the effect of a flow inhibitor on electroosmotic flow that could be used to extrapolate predictable qualitative and quantitative separation and resolution of peaks attributable to HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin if present in a blood sample. Shihabi teaches that “Hb C behaves similarly to hemoglobin HbA₂ in migration in this method” and that “[h]emoglobin E . . . comigrated with Hb C and also with HbA₂”. Ex. 1011 at 1011-0003. Shihabi itself teaches that different voltages affecting electroosmotic flow result in different haemoglobins being resolved. Shihabi provides no direct relationship between electroosmotic flow and any of the claimed haemoglobin variants, such that use of a flow inhibitor would predictably resolve HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin when present in a blood sample.

136. Camilleri teaches that “the separation of protein species varying only in subtle structural differences would be difficult, if not impossible, to achieve.” Ex. 1020 at 1020-0002. Coufal, unrelated to separation of any proteins let alone hemoglobins, teaches that “[i]t is difficult to control EOF through fused silica capillaries satisfactorily; EOF is very often unstable over a period of time.” Ex. 1013 at 1013-0001. Landers expressly states that any effect 1,4-diaminobutane may have to reduce EOF and enhance resolution of proteins “appears to be specific to glycoproteins, at least for the case of ovalbumin and pepsin.” Ex. 1026 at 1026-0008. Landers further states that “separation of a similar nonglycosylated protein *is not enhanced under the same conditions*” (Ex. 1026 at 1026-0009) thereby demonstrating unpredictability of the use of a flow inhibitor based on the nature of the protein. Accordingly, there is no scientific reason provided by Petitioner to justify that adding a flow inhibitor to the method of Shihabi would predictably result in separation and resolution of HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin when present in a blood sample. In my opinion, any results are empirical and are not predictable.

137. In addition, there is no basis for Dr. Wu’s conclusory statement that “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.” Ex. 1004, ¶104. Camilleri teaches that separation of structurally-close proteins is unpredictable. Coufal teaches that controlling EOF is unpredictable. Landers teaches that separation of glycosylated proteins versus similar non-glycosylated proteins using 1,4-diaminobutane is unpredictable. Accordingly, there is no reasonable expectation as evidenced by the art relied on by Petitioner that a flow inhibitor would separate and resolve hemoglobins in general and, in particular, separate and resolve HbA₂ and HbC, HbD, HbE, HbS, HbF or HbA, when present in a blood sample.

138. Huang teaches separation of haemoglobins A, F, S and C using “a neutral, hydrophilic coated capillary with no electroosmotic flow.” Ex. 1015 at 1015-0001 (“This value is two orders of magnitude less than the EOF of bare fused silica capillaries, and can be regarded as zero for normal CZE separations.”) Yet, Petitioner asserts that one of skill would nonetheless be motivated to add a flow inhibitor to reduce electroosmotic flow. Petitioner is clearly wrong. Huang operates with “no electroosmotic flow.” Accordingly, adding a flow inhibitor to reduce EOF would make no sense, and with the appropriate flow inhibitor, might even reverse the EOF, which was taught by Sunzeri, and over which the Examiner allowed the application. Further, there is no scientific reason provided by Petitioner to justify that adding a flow inhibitor to the method of Huang would predictably result in

separation and resolution of HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin when present in a blood sample. In my opinion, any results are empirical and are not predictable.

F. Ground 2: Claims 15 and 16 Depend from Claim 1 and Camilleri Does Not Provide Teachings Lacking in Shihabi or Huang and Coufal

139. Dependent claims 15 and 16 are directed to specific zwitterionic buffers and depend from claim 1. As discussed above with respect to claim 1, the combination of Shihabi or Huang and Coufal fails to teach (1) capillary electrophoresis using a blood sample, and (2) separating and resolving hemoglobin HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA hemoglobin. In addition, as set forth above with respect to claim 1, Petitioner has failed to demonstrate any scientific basis for asserting that adding a flow inhibitor to Shihabi or Huang would predictably result in separation and resolution of HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA hemoglobin when present in a blood sample. Camilleri provides no teaching that would cure the above deficiencies of Shihabi or Huang and Coufal.

G. Ground 3: Claims 4, 5, 8, 9 and 19-22 Depend from Claim 1 and Landers Does Not Provide Teachings Lacking in Shihabi or Huang and Coufal. Shihabi or Huang and Coufal and Landers Does Not Render Claim 24 Obvious.

140. Dependent claims 4, 5, 8, 9 and 19-22 are directed to various aspects of flow inhibitors and depend from claim 1. As discussed above with respect to claim

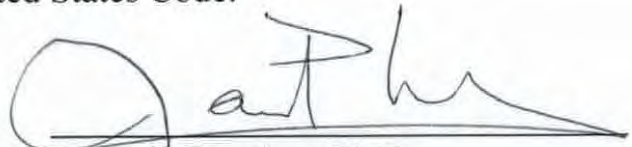
1, the combination of Shihabi or Huang and Coufal fails to teach (1) capillary electrophoresis using a blood sample, and (2) separating and resolving hemoglobin HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA hemoglobin. In addition, as set forth above with respect to claim 1, Petitioner has failed to demonstrate any scientific basis for asserting that adding a flow inhibitor to Shihabi or Huang would predictably result in separation and resolution of HbA₂ and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA hemoglobin when present in a blood sample. Landers provides no teaching that would cure the above deficiencies of Shihabi or Huang and Coufal.

141. As discussed above at ¶¶120-121 with respect to Ground 1 claim 23, Shihabi or Huang and Coufal each fail to teach or suggest a free solution capillary electrophoresis method using a blood sample, as recited in claim 24. Landers, likewise, does not teach using a blood sample and so provides no teaching that would cure the above deficiencies of Shihabi or Huang and Coufal. Accordingly, claim 24 is not obvious in view of Shihabi or Huang and Coufal and Landers.

142. Furthermore, as discussed above at ¶¶129-138 with respect to Ground 1 claim 23, there is no reasonable expectation as evidenced by the art relied on by Petitioner that a flow inhibitor would separate and resolve hemoglobins in general when present in a blood sample, as recited in claim 24. Accordingly, claim 24 is not obvious in view of Shihabi or Huang and Coufal and Landers.

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.

Dated: July 25/2024


James P. Landers, Ph.D.

APPENDIX A – SUMMARY AND OPINION OF ‘686 PATENT PROSECUTION HISTORY

1. The ‘686 patent issued from US application 11/280,025 (“the ‘025 application”) filed November 16, 2005. Ex. 1001 at 0001. The ’025 application claims the benefit of the filing date of French application 04 12263 filed November 18, 2004. *Id.*

2. The ‘686 patent indicates that the reference articles identified by the Patentee at col. 1 line 49 to col. 2 line 4 were provided to the United States Patent Office on an information disclosure statement for review by the Examiner during prosecution, namely Ishioka, *Biomedical Chromatography*, vol. 1, pp. 224-226, 1992; Sahin, *Journal of Chromatography A*, vol. 709, pp. 121-125, 1995; Jenkins, *Journal of Chromatography B*, vol. 682, pp. 23-34, 1996; Jenkins, *J. Cap. Elec.*, vol. 004, No. 3, pp. 137-143, 1997; Jenkins, *Clinica Chimica Acta*, vol. 289, pp. 121-132, 1999; Shihabi, *Electrophoresis 2000*, vol. 21, pp. 749-752, 1999; and Hempe, *Electrophoresis 2000*, vol. 21, pp. 743-748. *Id.* at 0016.

3. In a Preliminary Amendment dated September 19, 2006, the Patentee presented new claims 29- 55. Ex. 1003 at 0202-0205. Independent claim 29 is a method claim. *Id.* at 0203. Independent claim 53 is a kit claim. *Id.* at 0205. Independent claim 29 reciting a method for separating haemoglobins by capillary

electrophoresis using a buffer including a zwitterionic compound and a flow inhibitor is presented below. *Id.* at 0203.

29. (new) A method for separating haemoglobins in a biological sample by capillary electrophoresis comprising:
introducing said biological sample into a capillary tube comprising a solution of an analysis buffer, wherein said analysis buffer is of a zwitterionic type and at least one flow inhibitor, and
electrophoresing said biological sample in said analysis buffer and said at least one flow inhibitor whereby said haemoglobins are separated by migration.

Dependent claim 33 listing haemoglobin A2 and various other haemoglobin forms in the alternative is presented below.

Id. at 0203.

33. (new) The method according to claim 29, wherein the haemoglobins are a haemoglobin A2, and a C, a D, an B, a S, a F and/or an A haemoglobin.

4. The Examiner issued a restriction requirement dated November 14, 2008 requesting restriction between method claims 29-52 and kit claims 53-55. *Id.* at 0227. In a Response to Restriction Requirement dated December 12, 2008, the Patentee elected the method claims 29-52 for search and examination. *Id.* at 0241.

5. The Examiner issued a first office action dated January 8, 2009. *Id.* at 0243. The Examiner rejected claim 33 as reciting haemoglobin B, when the specification recites haemoglobin E. *Id.* at 0249. Pending claims 29-31, 34-36 and 43-52 were rejected under 35 U.S.C. 102(b) as being anticipated by Nouadje U.S. 2002/0162744. *Id.* With regard to claim 29, the Examiner stated that Nouadje

taught capillary electrophoresis for analyzing normal or abnormal blood proteins using a buffer system including a zwitterionic compound. *Id.* at 0250. With respect to then pending claims 47 and 48, the Examiner stated that Nouadje taught a sodium hydroxide pH modifying compound. *Id.* at 0251. The Examiner also believed that Nouadje taught the claimed flow inhibitor by describing an “additive that is capable of hydrophobic interaction with one or more protein constituents and capable of providing said protein constituents with one or more negative charges thereby reducing the electrophoretic mobility of said protein (page 1, section [0007])” and separating and detecting the protein constituents. *Id.* at 0250.

6. At page 10 of the office action, the Examiner rejected claim 33 under 35 U.S.C. 103(a) as being obvious over Nouadje in view of Chen U.S. Patent No. 5,202,006. *Id.* at 0253. The Examiner recognized that Nouadje ***failed to teach*** “the specific haemoglobin A2, and a C, a D, an B, a S, a F and/or an A haemoglobin.” *Id.* The Examiner stated that “Chen recognizes the use of capillary electrophoresis of haemoglobin A, A2, F which comprise normal adult blood (col. 1, lines 26-30), and haemoglobin S (col. 2, lines 1-5).” *Id.* at 0253-0254. The Examiner was also aware that Chen taught separation of HbA, HbF, HbS, and HbC using CZE and a barbituric acid buffer, by virtue of this teaching of Chen in the ‘686 patent at col. 2 lines 1-5. Ex. 1001 at 0016. However, the Examiner chose not to rely on Chen’s separation of HbA, HbF, HbS, and HbC in rejecting the claims.

7. Chen, therefore, teaches separation of only a subset of the claimed haemoglobins, namely HbA, HbF and HbS and HbC using a barbituric acid buffer and electroosmotic flow. While recognizing that HbA₂ is a component of normal haemoglobin, Chen provides no teaching of separating HbA₂ from other haemoglobins.

Nevertheless, the Examiner concluded at page 11 of the office action that,

Consequently, a person of ordinary skill in the art would accordingly have recognized the suitability, reasonable expectation of success, and subsequent predictability of utilizing capillary electrophoresis in effectively separating and analyzing the above haemoglobins.

The prior art can be modified or combined to reject claim as *prima facie* obvious as long as there is a reasonable expectation of success. See *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986) (see MPEP § 2143.02).

Furthermore, the claimed method involves applying the known technique of capillary electrophoresis to a known product comprising haemoglobin to yield the predictable result of facilitating effective separation and subsequent analysis as shown by the cited prior art. The Supreme Court has decided that applying a known technique to a known product ready for improvement to yield predictable results is likely to be obvious. See *KSR International Co. v. Teleflex.*, 550 U.S. ____, ____, 82 USPQ2d 1385, 1395-97 (2007) (see MPEP § 2143, D.).

Ex. 1003 at 0254.

Based on the above rationale, the Examiner concluded:

Therefore, it would have been obvious to a person of ordinary skill in the art to use haemoglobin variants such as A₂ and a C, a D, an B, a S, a F and/or an A.

Id.

8. However, the Examiner provided no analysis of the various forms of haemoglobin or how they may react to certain buffers in a capillary electrophoresis method. The Examiner provided no analysis of certain flow inhibitors and how certain analytes would predictably react to certain flow inhibitors. The Examiner provided no factual or scientific explanation on which to conclude reasonable expectation of success or predictability. The Examiner simply cited case law. The Examiner provided no basis on which one of skill could extrapolate Chen's results separating HbA, HbF and HbS and HbC using a barbituric acid buffer and electroosmotic flow to separating HbA₂ and also separating other forms of haemoglobins, such as HbC, HbD, HbE, and HbS, especially where HbC and HbE are structurally closely related to HbA₂ and where HbD and HbS are structurally closely related to each other. The Examiner's failure to provide any factual or scientific basis supporting the conclusion of a reasonable expectation of success is even more glaring in view of the express description in the '686 patent of the insufficiencies of prior art methods to separate haemoglobins, including Chen U.S. 5,202,006 cited by the Examiner and Shihabi.

9. The Examiner rejected claims 37-42 under 35 U.S.C. 103(a) as being obvious over Nouadje in view of Grover US 6,083,372. *Id.* at 0254-0255. The Examiner recognized that Nouadje failed to teach a specific flow inhibitor, but that

Grover taught various flow inhibitors including “1,4-diamino butane (col. 11, line 15).” *Id.* at 0255. The Examiner concluded that

[I]t would have been obvious to one skilled in the art to use various polyamine compounds such as 1,4-diaminobutane as a flow inhibitor for separating haemoglobin by capillary electrophoresis.

Id.

Again, the Examiner provided no factual or scientific explanation on which to conclude that one of skill would have had a reasonable expectation that 1,4-diaminobutane could be successfully used as a flow inhibitor to separate complex haemoglobins by capillary electrophoresis.

10. In an amendment dated April 8, 2009, the Patentee amended claim 1 to recite a “free solution capillary electrophoresis” method of a “blood sample.” Amended claim 29 is presented below.

29. (currently amended) A free solution capillary electrophoresis method for separating 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 a zwitterionic type and at least one flow inhibitor, and

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

Id. at 0296.

The Patentee also amended claim 33 to delete “B”, include “E” and clarify the nomenclature for the claimed haemoglobins. Amended claim 33 is presented below.

33. (currently amended) The method according to claim 29, wherein the haemoglobins are a haemoglobin HbA2, and an HbC, an HbD, an BHbE, an HbS, an HbF and/or an HbA haemoglobin.

Id.

The Patentee also added new claims 56 and 57 which are presented below.

56. (new) A free solution capillary electrophoresis method for separating 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 haemolyzed 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 a 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.

57. (new) A free solution capillary electrophoresis method for separating 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 a 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.

Id. at 0299-0300.

11. In response to the Examiner's anticipation rejection of claim 29 based on Nouadje, the Patentee argued that Nouadje taught serum proteins in serum

samples, such as albumin and globulins, and failed to teach a capillary electrophoresis method to separate haemoglobins in blood samples. *Id.* at 0306.. The Patentee further argued that Nouadje failed to teach that “a flow inhibitor will separate the various haemoglobin variants in blood samples, precisely because Nouadje taught separation of serum proteins such as albumin and globulin in serum samples”, and that serum does not include haemoglobin. *Id.* at 0307.

12. In response to the Examiner’s obviousness rejection of claim 33 based on Nouadje in view of Chen, the Patentee again argued that Nouadje failed to teach a method for separating haemoglobins or the use of a flow inhibitor for separating haemoglobins. The Patentee argued that Chen “merely defines some haemoglobin variants and their separation by CE in the presence of barbituric acids. There is no mention of the analysis buffer of the invention comprising a zwitterionic buffer and a flow inhibitor.” *Id.* at 0309. The Patentee concluded that “one of skill would not be motivated to combine the techniques disclosed in Nouadje et al. (albumin/globulin separation without flow inhibitor) together with Chen (haemoglobin variants) to arrive at the claimed invention, nor do these references, either alone or in combination, provide the reasonable expectation of success and subsequent predictability necessary to render the claimed subject matter *prima facie* obvious.” *Id.*

13. In response to the Examiner's obviousness rejection of claim 37 based on Nouadje and Grover, the Patentee argued "that Nouadje et al. fails to teach the separation of any haemoglobin variant in blood samples, let alone that a flow inhibitor may be used for this purpose." *Id.* at 0309-0310. The Patentee further argued that Grover taught electrokinetic chromatography (EKC), using a capillary filled with a stationary phase and an electrolyte) which is a different method from free solution capillary electrophoresis (FSCE). "Also, the electrokinetic chromatography (EKC) technique disclosed in the cited reference is carried out in a micellar phase with a surfactant in the buffer. These techniques are completely different from FSCE." *Id.* at 0310. Finally, the Patentee argued that Grover taught "the separation of phenols, verapamil and metopropol which are completely different from haemoglobins and thus cannot fairly be compared with haemoglobins" and, accordingly, that the buffer system of Grover was different from the claimed buffer system. *Id.* The Patentee concluded:

Simply put, one of skill in the art could not fairly find Applicants' claimed invention, including the use of various polyamine compounds as flow inhibitors suitable for use in the claimed methods, predictable or obvious given the limited disclosures of the two cited references.

Id.

Essentially, the Patentee argued that Grover taught a different method to separate different analytes, which could not be extrapolated to the claimed method.

14. In a final office action dated June 22, 2009, the Examiner rejected claim 29 under 35 U.S.C. 103(a) as being obvious over Nouadje in view of Alter US 5,753,094. *Id.* at 0347-0348. The Examiner repeated the rejection based on Nouadje, and apparently did not agree with Patentee's arguments that Nouadje was limited to serum proteins in serum samples, such as albumin and globulins, and failed to teach a capillary electrophoresis method to separate haemoglobins in blood samples. The Examiner recognized that "Nouadje et al. does not teach the use of blood, but does teach the use of protein serum which is a component of blood. Alter et al. teach that whole blood, serum, plasma, cerebrospinal fluid and urine can be separated and detected using electrophoresis (col. 3, lines 51-53)." *Id.* at 0348. The Examiner reasoned at page 5 of the office action that one of skill would use the method of Nouadje with a blood sample as taught by Alter

[B]ecause blood is a larger sample base than its component serum. It is known serum is a component of blood. It is also known that using a larger sample base, such as blood which contains the two major protein groups: albumin and globulin, will provide a broader range of data than just separating a component of blood such as serum.

Id. at 0348.

15. The Examiner rejected claim 32 (reciting the blood sample being haemolyzed blood) under 35 U.S.C. 103(a) as being obvious over Nouadje and Alter in view of Mohammad et al., Clinical Application of Capillary Isoelectric Focusing on Fused Silica Capillary for Determination of Hemoglobin Variants, Clinical

Chemistry 1997, 43: 1798-1799. *Id.* at 0350. The Examiner stated that Nouadje taught a method for separating haemoglobins using capillary electrophoresis, but did “not teach separation of haemolyzed blood or that the hemoglobin HbA₂ and an HbC, an HbD, HbE, an HbS, an HbF or an HbA hemoglobin.” *Id.* The Examiner cited Mohammad for teaching “the use of hemolyzed blood in capillary electrophoresis for identification of hemoglobin variants such as: C, S, F, and A.” *Id.* The Examiner concluded at page 7 of the office action that one of skill would use hemolyzed blood in the method of Nouadje because Mohammad teaches

[H]emolyzed blood is found within the red blood cells and disrupting the plasma membranes of the red blood cells would lead to faster and more accurate measurements.

Id.

The Examiner did not address the separation of HbA₂ from other hemoglobin variants, presumably because neither Nouadje nor Mohammad teaches HbA₂ separation from other haemoglobin variants.

16. The Examiner rejected claim 33 (reciting the seven haemoglobin variants) under 35 U.S.C. 103(a) as being obvious over Nouadje, Alter and Chen US 5,202,006. The Examiner reasoned that one of skill would look to Chen’s separation of normal haemoglobins A, A₂, and F

[B]ecause it is well known that hemoglobin HbA₂, A and F comprise the majority of hemoglobin contained in normal adult blood and it is known that in order to account for abnormalities in any scientific test,

one must have a standard base (i.e. normal hemoglobin) in order to detect the abnormalities.

Id. at 0350-0351.

Again, the Examiner did not rely on Chen's teaching of separating HbC, HbS, HbF and HbA using a barbituric acid buffer. Again, the Examiner provided no factual or scientific explanation of why one of skill would reasonably expect the prior art methods to result in separation of HbA₂ from other various forms of haemoglobin, such as HbC and HbE, especially when the '686 patent taught that prior art methods failed to achieve such separation, including Chen and Shihabi.

17. The Examiner rejected claim 37 (reciting specific flow inhibitors) under 35 U.S.C. 103(a) as being obvious over Nouadje, Alter and Grover US 6,083,372. *Id.* at 0351. The Examiner maintained the position that flow inhibitors are commercially available, Grover teaches the specific flow inhibitor 1,4-diaminobutane, and that selection of a known material "based on its suitability for the intended use, is within the ambit of one of ordinary skill in the art." *Id.* The Examiner also states that "the combination of familiar elements is likely to be obvious when it does no more than yield predictable results." *Id.* at 0352. Finally, the Examiner concluded "it would have been obvious to one skilled in the art to use various polyamine compounds such as 1,4-diaminobutane as a flow inhibitor for separating haemoglobin by capillary electrophoresis." *Id.* at 0351-0352.

18. However, the Examiner provided (1) no explanation of why one of skill would reasonably expect that a specific flow inhibitor for use with the chemicals of Grover would function as a flow inhibitor to separate complex haemoglobins with slight differences between variants. The Examiner responded to the Patentee's arguments that one of skill would not be motivated to combine Nouadje and Chen by stating that

[T]he Court's have held the combination of familiar elements is likely to be obvious when it does no more than yield predictable results. Furthermore, the combination of a known technique to a product is likely to be obvious when predictable results are achieved. . . . Therefore, the motivation provided by the examiner is that applicant used a known technique with a known product.

Id. at 0352-0353.

However, the Examiner again failed to provide any factual or scientific explanation supporting that Nouadje's method would be understood in view of Chen to separate and resolve haemoglobins with a reasonable expectation of success. The Examiner simply concluded "predictable results" by alleging elements of the claims within various prior art references based on hindsight.

19. In an Amendment and Response dated December 22, 2009, the patentee amended claim 29 to recite separating haemoglobin HbA2 and an HbC, an HbD, an HbE, an HbS, an HbF or an HbA haemoglobin from claim 33 and cancelled claim 33. *Id.* at 0396. Amended claim 29 is presented below.

29. (currently amended) A free solution capillary electrophoresis method for separating 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 a 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.

Id.

20. Importantly, claim 29 was amended to require separation HbA2 and at least one of the remaining six recited haemoglobin variants, i.e. by virtue of the use of the word “or”. *Id.* Accordingly, claim 29 does not require all seven variants to be present in a blood sample to be separated. The Patentee explained that claim 29 was amended “to specify the haemoglobin variants separated according to the method of the present invention.” *Id.* at 0401. The ‘686 patent demonstrates that all seven variants can be separated when present in a sample using the claimed zwitterionic buffer and a flow inhibitor.

21. In response to the rejection of claim 29 based on Nouadje and Alter, the Patentee argued that Nouadje taught separation of “seric proteins” and that such “seric proteins” do not include haemoglobin variants. *Id.* at 0402. The Patentee further argued that “seric proteins have physical and chemical properties different from those of haemoglobins. The respective natures of seric proteins and

haemoglobins have an impact on the principle of separation.” *Id.* at 0402. The Patentee explained further that

(1) A seric protein differs from the other seric proteins by their entire structure. A seric protein can be shifted and therefore better separated from other seric proteins by using specific additives changing the ratio charge/weight can be used. Amongst these additives, due to the specific hydrophobicity of a seric protein, additive having a hydrophobic portion can be used (see Nouadje).

Id.

Accordingly, the Patentee argued that Nouadje taught use of an additive to alter the charge of the analyte, and therefore the charge/weight ratio, to thereby shift migration of the analyte leading to better separation. The Patentee explained that “haemoglobin variants are difficult to distinguish from each other because they can vary by only one amino acid, the remaining part of the molecule being exactly the same as in the other variants. The variants are distinguished from each other by their isoelectric point.” *Id.*

22. The Patentee further explained that haemoglobins are structurally different from seric proteins and that the principle of separation of seric proteins is different from that of haemoglobins. For seric proteins, an additive having a hydrophobic portion is used to “interact with the hydrophobic portion of the seric protein” creating a “shift of one specific peak corresponding to a specific globulin.” *Id.* at 0403. In contrast, the claimed flow inhibitor “acts on all the variants” to improve resolution. *Id.*

When an additive is added to a blood sample for improving the separation of Hb variants, the variants remain in the same order when separated. In other words, the transformation of the profile is homothetic. To the contrary, the seric protein peaks in an electrophoregram are shifted under the influence of the additives having an influence on the ratio charge/weight

Id. at 0403 (emphasis original).

The Patentee again asserted that Nouadje did “not teach the separation of blood samples, but of seric proteins.” *Id.* at 0404. In addition, Nouadje fails to teach that the resolution of haemoglobin variants can be improved.” *Id.*

23. The Patentee argued that Alter taught “a storage buffer or diluent, beside the running buffer (borate).” *Id.* The Patentee acknowledged that the sample can be blood but that “no Hb variant resolution is suggested nor carried out according to Alter.” *Id.* The Patentee explained that the analysis of haemoglobin variants from a blood sample requires (1) a surfactant to lyse the red blood cells to release the haemoglobin or (2) a diluent conductivity that is low. *Id.* at 0404-0405. The Patentee explained that Alter did not teach use of a surfactant and so did not teach a blood sample that can be used for analysis of haemoglobins.

If no lysis of blood occurs, no Hb is in solution and as a consequence, the analysis of Hb variants is not possible.

Id.

The Patentee also explained that Alter discloses use of a diluent with a high conductivity, and “as a consequence, Applicants submit that Alter cannot be

considered as adapted for the resolution of Hb variants.” *Id.* The Patentee concluded that

[I]t would not have been obvious to one skilled in the art to modify the method of Nouadje to utilize blood as the biological sample of interest in view of the teachings of Alter and predictably arrive at Applicants’ method of Hb variant resolution as claimed in the instant invention.

Id. at 0405.

Since the subject matter of claim 33 was introduced into claim 29, the Patentee argued that Chen teaches that “only poor resolution (separation) of the principle haemoglobin variants have been achieved in the past by prior art methods and discloses a separation method comprising the use of a buffer comprising barbituric acid”, and that Chen does not “teach or suggest the zwitterionic buffer disclosed in Applicants’ invention.” *Id.* at 0405-0406. The Patentee concluded that “the combination of the elements disclosed in Nouadje, Alter and Chen do not predictably yield the methods of the instant invention” *Id.* at 0406-0407.

24. In response to the rejection of claim 32 (reciting hemolyzed blood), the Patentee acknowledged that Mohammad “relates to the separation of some Hb variants.” *Id.* at 0405. But the Patentee argued that Mohammad taught a run buffer of ampholine in a methyl cellulose solution which was different from the claimed buffer including a zwitterionic compound and a flow inhibitor. *Id.* at 0406. Under its buffer conditions, Mohammad was “unable to consistently resolve haemoglobin C from A₂ and E.” *Id.* The Patentee distinguished the claimed invention from that

of Mohammad because the claimed invention resolves HbA₂, and at least one of HbC and HbE using the claimed combination of a zwitterionic compound and a flow inhibitor, which Mohammad was unable to accomplish using its buffer. *Id.* at 0405-0406.

25. In response to the rejection of claim 37 (describing certain flow inhibitors) based on Nouadje, Alter and Grover, the Patentee argued that Grover describes using electrokinetic chromatography (EKC) to separate phenols, verapamil and metoprolol which “cannot fairly be compared to Hb variants.” *Id.* at 0407. The Patentee emphasized that the “technical field of Hb variants separation is a specific technical field” and that Grover is directed to the different technique of electrokinetic chromatography (EKC). *Id.* at 0408. Even though Grover discloses using 1,4-diaminobutane, the Patentee argued that “it would not have been obvious to one skilled in the art to use 1,4-diaminobutane as a flow inhibitor for separating haemoglobin by capillary electrophoresis as claimed in the instant invention.” *Id.* In sum, the Patentee argued that the separation techniques are different between Grover and the claimed invention (EKC v. CE) and the species to be separated are different between Grover and the claimed invention (phenols, verapamil and metoprolol v. Hb variants) and so one of skill had no reasonable expectation of successfully using 1,4-diaminobutane to separate HbA₂ from the other recited haemoglobins. *Id.* at 0407-0408.

26. The Examiner issued a nonfinal rejection dated January 29, 2010 withdrawing all the rejections of record based on Nouadje, Alter, Chen and Grover. *Id.* at 0414. The Examiner rejected claims 29-32, 34-36, 43, 49, 50 and 56 under 35 U.S.C. 102(b) as being anticipated by newly cited Sunzeri US 5,536,382 with evidence from Camilleri, Capillary Electrophoresis, 1997. *Id.* at 0415.

27. Regarding claim 29, the Examiner alleged that Sunzeri taught a capillary electrophoresis method “for separating and detecting (claim 30) hemoglobin in a lysed (i.e., haemolyzed) blood sample (claims 31 and 32) utilizing a plain capillary and a 50mM tricine buffer (col. 11, lines 25-50, claims 34-36).” *Id.* Sunzeri, therefore, taught capillary electrophoresis of a blood sample, which Nouadje failed to teach.

28. The Examiner alleged that tricine is the claimed zwitterionic compound. *Id.* at 0417. The Examiner noted that Sunzeri taught separation of HA1c. *Id.* The Examiner did not explain how Sunzeri taught separation and resolution of more than one haemoglobin (claims 56 and 57). The Examiner certainly did not explain how Sunzeri taught separation and resolution of HbA₂ and also separation and resolution of at least one of the remaining six haemoglobin variants HbC, HbD, HbE, HbS, HbF or HbA, as recited in claim 29.

29. The Examiner also alleged that Sunzeri taught the claimed flow inhibitor (the positively charged quaternary amine compound CTAB) insofar as Sunzeri described the concept of “electroosmotic flow” and that

[A] quaternary amine (i.e. CTAB) can be used for ***electroosmotic flow reversal*** (col. 8, lines 13-15, i.e. giving “flow inhibitor” its broadest reasonable interpretation)(see Camilleri, Capillary Electrophoresis, which evidences that CTAB can be added to a buffer for capillary electrophoresis ***in order to reverse charge on the silica capillary surface, thus creating a positively charged surface***, see page 371).

Id. at 0415 (emphasis added).

As understood by one of skill in the art, electroosmosis from the anode to the cathode is referred to as “normal electroosmotic flow”, and electroosmotic flow from the cathode to the anode is referred to as “reverse electroosmotic flow”. The Examiner provided no factual or scientific explanation of how reversing the electroosmotic flow would result in separation of haemoglobins in general and, in particular, separation of HbA₂ and also separation of at least one of the remaining six haemoglobin variants HbC, HbD, HbE, HbS, HbF or HbA. Further, the Examiner referred to no experimental results in Sunzeri using a buffer including both the zwitterionic compound and the Examiner’s alleged flow inhibitor CTAB.

30. Despite the Examiner’s allegation that Sunzeri taught both a zwitterionic compound and a flow inhibitor, Sunzeri does not teach separation and resolution of more than one haemoglobin and certainly not separation and resolution

of HbA₂ and also separation and resolution of at least one of the remaining six haemoglobin variants HbC, HbD, HbE, HbS, HbF or HbA, as claimed.

31. The Examiner rejected claims 37-42, 45, 46 and 57 under 35 U.S.C. 103(a) as being obvious over Sunzeri in view of Camilleri with evidence from Hempe et al. (*Capillary isoelectric focusing of hemoglobin variants in the pediatric clinical laboratory*, Electrophoresis 1997, 18, 1785-1795). *Id.* at 0417. The Examiner recognized that Sunzeri did not “teach the use of a flow inhibitor of an aliphatic or cyclic diamine or a polyamine type (i.e. 1,4 diaminobutane).” *Id.* at 0418. Instead, the Examiner relied on Camilleri alleging that Camilleri

[T]eaches that capillary electrophoresis can be performed without chemically modifying the capillary using a zwitterionic buffer and additives such as 1,4 diaminobutane, with concentrations of up to 60mM, which will suppress capillary surface interactions (page 370) because the amine will compete with the negatively charged protein for absorption sites on the capillary wall (page 370)(i.e. a hemoglobin species that contains a net negative charge will have the same capillary surface interaction as a negatively charged protein).

Id. at 0418.

The Examiner concluded obviousness as follows.

Therefore, it would have been obvious to one skilled in the art to modify the buffer of Sunzeri to contain the flow inhibitor 1,4 diaminobutane as taught by Camilleri because the flow inhibitor will function to suppress capillary surface interactions of the negatively charged hemoglobin by competing for absorption sites on the capillary wall thus modifying the charge on the silica capillary surface creating a positively charged surface which will reverse or inhibit electroosmotic flow.

Id.

32. However, the Examiner provided no explanation of how a molecule like 1,4-diaminobutane will be expected to create a positively charged capillary surface wall like the CTAB of Sunzeri so as to reverse or inhibit electroosmotic flow. The Examiner provided no explanation of why one of skill would expect the 1,4-diaminobutane would be interchangeable with the CTAB of Sunzeri. Further, the Examiner did not provide any explanation of why the use of 1,4 diaminobutane of Camilleri in the method of Sunzeri would lead one of skill to reasonably expect separation and resolution of more than one haemoglobin or separation and resolution of HbA₂ and also separation and resolution of at least one of the remaining six haemoglobin variants HbC, HbD, HbE, HbS, HbF or HbA, as claimed. Sunzeri only discloses separation of HA1c using a zwitterionic buffer. Again, Sunzeri provides no experimental results using both a zwitterionic compound and the alleged flow inhibitor CTAB.

33. In response to the Examiner's non-final rejection, the Patentee filed an amendment dated July 21, 2010. *Id.* at 0452-0465. Regarding the rejection of claims 29-32, 34-36, 43, 49, 50 and 56 as being anticipated by Sunzeri with evidence from Camilleri, the Patentee argued that Sunzeri taught use of a labeled reagent ("binding pair member") to bind to an analyte "and then employing capillary electrophoresis to resolve the bound from unbound reagent. The sample constituents are then quantitated by measuring directly or indirectly, the bound reagent." *Id.* at 0458. The

Patentee argued that Sunzeri taught that the reagent bound to the target analyte is resolved based on the “reagent [being] displaced from its normal migration position” as a result of being bound to the target analyte. *Id.* at 0458-0459. Sunzeri teaches antibody and target antigen as binding pair members, and in particular, a labeled antibody mouse anti-human hemoglobin A1c as the antibody specific for hemoglobin A1c. *Id.* at 0459.

34. The Patentee argued that Sunzeri did not anticipate the claimed method because Sunzeri taught “detection of a single hemoglobin variant” using a labeled binding reagent, and that “the claims of the present invention are directed to separating multiple hemoglobin variants in a biological sample using capillary electrophoresis.” *Id.* (emphasis in original). “Indeed, the only separation taught in Sunzeri is the resolution of bound from unbound reagent”, namely mouse anti-human hemoglobin A1c monoclonal antibody-hemoglobin A1c complexes from unbound antibody. *Id.* at 0459-0460.

Moreover, Applicants respectfully point out that Sunzeri’s method only permits the analysis of a single constituent in a biological fluid per electrophoretic run; the detection and separation of more than one analyte-reagent complex is not taught. In contrast, ***Applicants’ invention successfully achieves the separation of multiple hemoglobin variants in a single electrophoretic run, i.e., hemoglobins HbA2, HbC, HbD, HbE, HbS, HbF and HbA. This separation of multiple hemoglobin variants is clearly depicted in Figures 1-7 of the instant application.***

Id. at 0460 (emphasis added).

35. The Patentee further distinguished Sunzeri based on the labeled reagent being specific for an analyte and being shifted or displaced from its normal migration position while the claimed method uses a flow inhibitor which interacts with the capillary and not the analyte.

In contrast, the methods of the instant invention ***comprise the use of an analysis buffer of the zwitterionic type which comprises at least one flow inhibitor***. 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 an homothetic manner, i.e., the variants remain in the same order when separated.

Id. at 0461 (emphasis added).

36. The Patentee also argued that Sunzeri teaches that the positively charged quaternary amine compound CTAB can be used for ***EOF reversal***. *Id.* at 0462. Likewise, Camilleri teaches that CTAB can be used to ***reverse*** the charge on the silica capillary surface from a negative charge to a positive charge. *Id.* In contrast, the Patentee argued that the claimed flow inhibitor “is used to *decrease* the charge density on the capillary surface, and thus *reduce* the EOF.” *Id.* at 0461.

Applicants submit that *reversal* of EOF by CTAB as disclosed in the prior art is not the same as *reduction* of EOF, and thus the art does not in fact teach or even suggest the use of CTAB as a flow inhibitor such as described in the methods of the present invention. Specifically, *Camilleri* teaches that one can reduced the protein adsorption on a capillary surface by increasing the concentration of CTAB in the buffer used during capillary electrophoresis. The addition of CTAB causes a reversal of charge on the capillary surface, thus creating a *positively* charged surface, which causes a reversal of the EOF. As disclosed by Applicants, however, the flow inhibitor is used to decrease the charge

density, and thus *reduce* the EOF – *not to reverse EOF*. Indeed, Applicants submit that hemoglobin variants have isoelectric points between about 6 and 7.7; thus at alkaline pH, hemoglobins are *negatively charged, as is the capillary surface*. If the capillary surface is positively charged, which is the case when reversal is caused, no separation would occur because of the interaction between the positively charged capillary surface and the negatively charged proteins.

Id. at 0461-0462.

37. Regarding the rejection of claims 37-42, 45, 46 and 57 as obvious over Sunzeri, Camilleri and Hempe, the Patentee again argued that Sunzeri and Camilleri “do not teach separation of hemoglobin variants.” *Id.* at 0463 (emphasis in original).

The Patentee further argued that

Significantly, *Sunzeri* also fails to disclose the actual claimed hemoglobin variants, nor does it teach or even hint at the possibility that one could separate these or even hint at the possibility that one could separate these or any other hemoglobin variants by free solution capillary electrophoresis. Indeed, since the methods taught by *Sunzeri* only permit the analysis of a single analyte at a time, the separation of multiple proteins is not even possible, nor is it even remotely contemplated, in this reference.

Id.

38. The Patentee distinguished Hempe from the claimed subject matter because it taught capillary isoelectric focusing (cIEF) which is a very different method for separating hemoglobins compared to free solution capillary electrophoresis. *See id.*

39. In response to Applicants' arguments, the Examiner issued a notice of allowance dated October 12, 2010 allowing claims 29-32, 34-51, 56 and 57 after making an Examiner's amendment to claims 29, 56 and 57 to insert "and resolving" after "separating". *Id.* at 0470-0473. The Examiner issued the following statement of reasons for rejection.

The instant application is allowable over other art ***because the instant application teaches a free solution capillary electrophoresis method for separating and resolving haemoglobins in a biological sample.*** The method comprises electrophoresing utilizing ***an analysis buffer that is a zwitterionic type and at least one flow inhibitor.***

The closest prior art of record, Sunzeri (US 5,536,382) teaches a method of capillary electrophoresis for separating and detecting haemoglobin in a lysed blood sample utilizing a tris tricine buffer. Sunzeri mixes the sample with a labeling reagent and detects the labeling reagent's displacement from its normal migration position from unbound reagent. Sunzeri teaches the detection of a single specific analyte, not multiple haemoglobins. In Sunzeri, only the resolution of bound from unbound reagent is taught. However, ***the present invention not only separates haemoglobins, it also provides for the resolving of the different haemoglobin components such as HbA2, HbC, HbD, etc.***

Additionally, Nouadje et al. (US 2002/0162744) was cited, however, while Nouadje et al. teach a method of electrophoresis for separating blood components which includes haemoglobins, Nouadje et al. does not specifically teach the resolution of the different haemoglobin constituents. Rather Nouadje et al. only teach the detection of haemoglobin in the sample, ***it does not resolve the different haemoglobin constituents.***

Id. at 0472 (emphasis added).

40. Accordingly, the Examiner understood that the art, including Shihabi and Camilleri, did not teach or suggest the combination of a zwitterionic buffer and a flow inhibitor to resolve different haemoglobin constituents in a blood sample. On January 3, 2011, the Patentee paid the issue fee resulting in issuance of US 7,887,686 on February 15, 2011. *Id.* at 0483-0485.