

SEPARATIONS

Keeping you on track with the latest in EP

Hemoglobinopathy & Thalassemia Detection: Traditional Methods and a Novel Method – Capillary Electrophoresis Technology

By: Aigars Brant, Ph.D., Scientific Affairs Officer

BACKGROUND

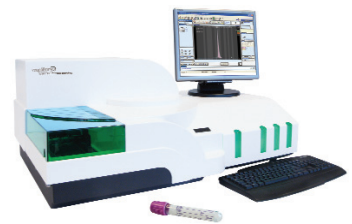
In humans, two pairs of unlike globin chains combine to form four heme groups to form hemoglobin (Hb), a protein that is carried by red cells and picks up oxygen in the lungs and delivers it to the peripheral tissues. One of the globin chain pairs in hemoglobin is always alpha (with the exception of the very first weeks of embryogenesis), while the second pair is "non-alpha", i.e., it can be made of beta- (β), delta- (δ), or gamma (γ) chains.

In the healthy newborn, Hb F ($\alpha_2\gamma_2$) is the major hemoglobin (~75%). Fetal hemoglobin (Hb F) is replaced by Hb A ($\alpha_2\beta_2$) and Hb A2 ($\alpha_2\delta_2$) during the first 6 to 12 months of life. In healthy adults, hemoglobin is comprised of Hb A (~97%) and Hb A2 (~2.7%), with only trace amounts of Hb F, if any.

Two types of disorders may affect globin chains – qualitative and quantitative. Qualitative disorders, i.e., hemo-globinopathies, result from any of the following: i) substitution of one amino acid for another (as in Hb S and Hb C), ii) deletion of a portion of the amino acid sequence (as in

Hb Gun Hill), iii) abnormal hybridization between two chains during meiosis (as in Hb Lepore), and iv) abnormal elongation of the globin chain (as in Hb Constant Spring).

Obviously, any alterations listed above lead to changes in molecule structure or charge and they can be detected with the appropriate methodology.



CAPILLARYS 2 FLEX Piercing – whole blood Hemoglobinopathy testing.*

Currently, 1,036 Hb variants are listed in the globin chain database with the majority of them being beta chain variants (746).

Thalassemias are quantitative disorders affecting the rate of otherwise normal hemoglobin synthesis. The β -thalassemia carrier state is a benign condition with mild anemia, red blood cell hypochromia and microcytosis, and an elevated Hb A2 level. In comparison, severe disease (β -thalassemia major) requires lifelong blood transfusions and chelation therapy.

Continued on pg 2

So why don't airplanes fall out of the sky?

By: E. Richard Hunt, MBA, National Service Manager

If your first thought runs to Bernoulli's principle – the aerodynamic theory that explains how lift is created – you've hit on one piece of the puzzle. However, thousands of electronic, hydraulic, and mechanical parts must also work in concert to keep a plane safely aloft. A malfunction in any of these components can cause major delays or, worse yet, down the plane. Averting such disasters requires more than a review of hypothetical models.

It is through the analysis of failure data, gathered from actual operating circumstances, that allows the aeronautics industry to identify problems before they blossom into catastrophes. Though we don't maintain aircraft, the Sebia service team is adopting many of these same practices to better address your needs.

The benefits are clear. Sebia can ensure instrument reliability and customer confidence by identifying the indicators of future problems and resolving them proactively. Such preventive measures not only reduce direct costs – excessive emergency calls, out-of-territory travel, etc. – but, most importantly, they minimize downtime and optimize patient care.

Equally as important, we must communicate and work together with you, our customers, to tailor preventive maintenance protocols which specifically address the unique challenges of your operating environment. This requires us to review more than the user logs; we must talk with your team and take note of their experiences.

It is imperative that we continually adapt our service models to meet and exceed your evolving needs. It is with these forward-looking practices – communication, partnership, and intelligent proactive actions – that we can ensure your success with Sebia products.

Continued from pg 1 - Hemoglobinopathy & Thalassemia Detection

Since increase in Hb A2 concentration is indicative of beta-thalassemia, it is useful to obtain an accurate relative Hb A2 value. The most common (beta chain) Hb variants can interfere with an accurate Hb A2 quantitation with many testing methods. It is also important to account for delta chain Hb variants in the sample; if present, the delta chain variant concentration (%) must be added to the Hb A2 value to obtain an accurate total Hb A2 concentration.

Alpha-thalassemia affects the synthesis of alpha globin chains and the severity of disease is dependent on the extent of gene deletion. Loss of two out of four alpha-chains encoding genes results in an α -thalassemia trait, characterized by microcytosis with little or no anemia. Loss of three genes results in Hb H (4 β chains) disease, a moderate hemolytic anemia, while loss of all four genes is incompatible with independent life.

ELECTROPHORETIC HEMOGLOBIN SEPARATION METHODS

Electrophoresis has long been the method of choice in hematological laboratories for qualitative and quantitative hemoglobin analyses. Currently, four different electrophoresis techniques are routinely used in the lab setting: 1) alkaline and acid gel electrophoresis, 2) isoelectric focusing (IEF), 3) high-pressure liquid chromatography (HPLC) and 4) capillary electrophoresis (CE).

Sebia Electrophoresis provides multiple platforms for the detection of hemoglobinopathies and thalassemias - fully automated capillary electrophoresis systems (CAPILLARYS™ 2 and MINICAP) and a semi-automated agarose gel system (HYDRASYS® 2) to accommodate both alkaline and acid agarose gel electrophoresis.

ALKALINE AND ACID AGAROSE ELECTROPHORESIS

Because of its simplicity, alkaline gel electrophoresis is one of the most popular methods for Hb screening. Semi-automated agarose gel electrophoresis is also cost-effective for low to medium volume laboratories. However, the technique is relatively laborious, requiring manual sample preparation. Red blood cells must be washed in saline to remove plasma proteins and to eliminate non-hemoglobin bands on the gel. Electrophoresis at alkaline pH (8.5) allows for the separation of the major hemoglobins and a number of less common Hb variants. Visualization of the Hb bands is done by automated staining of the gel with amido black. The clear background of the gels enables measuring the concentration of individual fractions by densitometric scanning. However, due to the precision and accuracy of Hb in low concentrations (Hb A2 for example), the College of American Pathologists (CAP) no longer recommends the use of densitometric scanning for quantification of Hb A2. With alkaline agarose gel testing, some common Hb variants comigrate, such as Hb C, Hb E, Hb A2 & Hb O-Arab and Hb S, Hb D & Hb G.

In order to separate some Hb variants that commonly comigrate at alkaline conditions, the sample may also be analyzed on gel at an acidic pH (6.0). In these conditions, molecular charge will differ and migration patterns will change. As a result, Hb S can be differentiated from Hb D, and Hb C can be differentiated from Hb E.

ISOELECTRIC FOCUSING

IEF provides excellent separation of many hemoglobin variants and detects fast migrating or low concentration hemoglobin variants such as Hb H, Hb Bart's, and delta chain variants as well. IEF gels contain special molecules - ampholytes - that create a pH gradient in an electrical field. When a pH gradient is present, hemoglobin molecules migrate to a position on the gel where the net charge equals zero (0), resulting in very narrow and focalized bands. On IEF gels, Hb C separates from Hb E and Hb O-Arab, and Hb S from Hb D and Hb G. IEF gels, however, are processed manually and require a significant amount of technical time. Additionally, IEF results are qualitative and interpreting results can be quite subjective from one technologist to another.

HIGH-PRESSURE LIQUID CHROMATOGRAPHY

HPLC is a pressure driven technique. Hemoglobin samples are injected into a resin column and retained based on the charge. The eluting solution that competes for the negatively charged resin is added in increasing concentration. Hemoglobin variants elute from the column and are detected at 415 nm, then at 690 nm to correct the baseline of the result. The hemoglobin retention time (from injection until the maximum point of each peak) is calculated and plotted on a chromatograph.

HPLC instruments are primarily indicated for the measurement of Hb A2 and F, but also provide data (retention times) on many Hb variants. However, HPLC should not be used as the sole method for identification of hemoglobin variants (1). HPLC is very complementary to CE technology; together these two automated methodologies provide valuable data for result interpretation (2) (3). HPLC techniques result in patterns that are relatively complex and require training and experience for interpretation of results (4).

The following table lists HPLC migration characteristics in the presence of common variants.

Hb present	Hb A2 result	Hb F result	Comments
S	falsely elevated		coelution of Hb S1c fraction with A2 (4)
E	falsely elevated		coelution of Hb E with A2 (4)
D	underestimated		(3)
G-Philadelphia	falsely elevated		coelution of G-Philadelphia with A2
Lepore	falsely elevated		coelution of Lepore with A2 (3)
A1c (elevated)		falsely elevated	(3)

CAPILLARY ELECTROPHORESIS (CE)

CE is the newest FDA-cleared method for the quantitation and detection of normal and abnormal hemoglobins, as an aid in the diagnosis of hemoglobinopathies and thalassemias. Sebia is the only company with FDA-cleared CE technology for hemoglobinopathy testing.

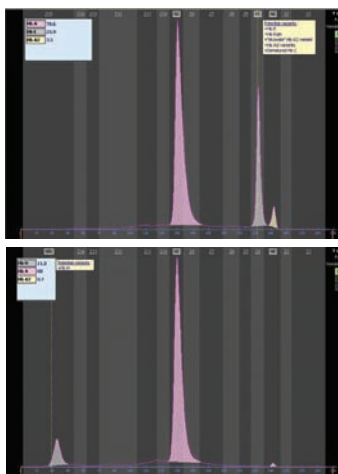
CE technology utilized liquid flow electrophoresis - buffer replaces agarose gel as the medium. Hemoglobin variants are separated with the principles of electroosmotic flow at an alkaline pH (9.4) with a negatively charged silica capillary support, and high voltage. Multiple samples undergo an 8-minute high-resolution separation, concurrently. A high-resolution hemoglobin separation is obtained, similar to IEF separation. The ideal wavelength of 415 nm is utilized for hemoglobin detection with CE. The result, or electropherogram, is made up of 300 consecutive readings (dots) and is divided into 15 zones. To facilitate interpretation, results are automatically positioned with regard to the Hb A fraction in the sample. Hemoglobins (normal and variant) are displayed as peaks and the zone to which a variant belongs is identified automatically by the system. An on-board hemoglobin library is present in the form of a dropdown list and lists all of the normal and variant hemoglobins that may be present within a particular zone.

The CAPILLARYS 2 and MINICAP, Sebia's automated CE platforms, utilize the same separation principle, reagents, and capillaries; however, they differ by the number of capillaries running simultaneously. The CAPILLARYS 2 instrument has eight capillaries, while the MINICAP instrument has two capillaries. Both CE systems provide continuous sample feed and complete traceability of results.

With Sebia's CE systems, packed red blood cell samples are utilized for analysis. Plasma is removed from samples and the bar-coded primary sample tube is loaded onto the instrument; all other steps in sample processing and separation are performed automatically by the system.

MORE ON CE TECHNOLOGY

High-Resolution Separation



- In one analysis, separation of Hb S from Hb D, and Hb C from Hb E (and from Hb A2).
- Precise, quick quantification of Hb F and Hb A2, even in the presence of Hb S (5). Posttranslational Hb variants (such as glycated HbS1c) do not separate from the main fractions (6).
- Superior to HPLC in the measurement of Hb A2 in the presence of Hb E (7).
- Delta chain variants, alpha chain variants, and other minor Hb fractions are readily visualized (8).
- Hb H and Hb Bart's are more readily detected and measured by CE than by the HPLC method (4).

Automation & Throughput

- Greater throughput in comparison to HPLC - 33 results/hour (CAPILLARYS 2) or 8 results/hour (MINICAP), making CE ideal for a screening methodology (8).

Multituse Instrument

- Sebia systems may be used for other types of analysis including: serum / urine protein electrophoresis, Immunotyping (automated immunofixation alternative), and CDT (a marker for chronic alcohol abuse) (8).

Continued on pg 4

CompuNet is Complimentary of CAPILLARYS 2

Hands-free capillary electrophoresis gives Ohio lab faster throughput, clear results

It's unlikely anyone would say that the rising demand for oncology services is a good thing—even if it's an indicator of increasing survival rates. Aside from wanting people to be well, healthcare providers struggle when patient volumes threaten their ability to provide timely diagnoses and care. CompuNet Clinical Laboratories faced such a challenge when its oncology practice client base grew—and tested the limits of the lab's electrophoresis capabilities.

In 2001, CompuNet was using a very hands-on method, which made it difficult to keep up with the increasing workload. That's when Sebia entered the picture. **"We discovered the HYDRASYS®, Sebia's semi-automated electrophoresis system, and found we could speed up our urine and serum protein and immunofixation (IF) processing with high quality results,"** explains Mark Shearer, MCLT, MT (ASCP), Director of Chemistry at the Dayton, Ohio-based lab.

"It's totally transparent—the technologist can load up a batch, start the system and turn to another task while the samples are processing."

So later, when Sebia introduced the fully automated CAPILLARYS™ 2 for hands-free capillary electrophoresis, Shearer and his colleagues were intrigued. The CAPILLARYS 2 provides complete "walkaway" automation, from bar-coded primary sample tube to final result: positive sample ID. After



Left to Right: Kathy Hall, Terri Grant, Debbie Swigart, Debra Popadyn, and Julie Ruggieri



Sandy Frommeyer and Dr. Daniel Hood

moving to the new instrument in March 2007, CompuNet was able to increase serum protein electrophoresis processing and immunotyping by 10.3 percent and 18.5 percent, respectively, from 2006 levels.

"It's totally transparent—the technologist can load up a batch, start the system and turn to another task while the samples are processing," notes Sandy Frommeyer, MT (ASCP), Chemistry Team Leader at CompuNet. CAPILLARYS 2 can achieve a throughput of 80 protein samples per hour.

Hands-free...but big-brained

One important advantage of the CAPILLARYS 2 is that it's error-proof—a critical concern in an environment that demands utmost consistency. Full sample traceability begins when the technologist loads bar-coded primary sample tubes in the CAPILLARYS sample rack. The instrument reads the barcodes and generates a work list for sample tracking.

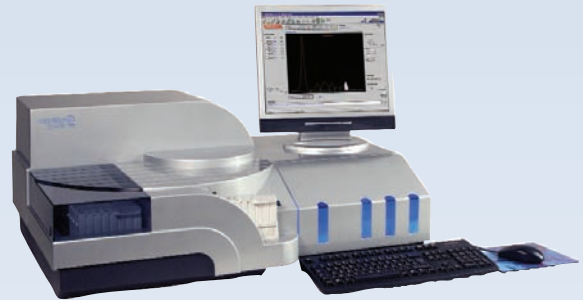
A very small amount of sample is utilized during processing. Here's a brief look at how the instrument works: The sample and buffer are introduced into eight capillaries contained on the instrument, each of which is in contact with an anodic and cathodic buffer reservoir. High voltage is applied. Protein migration and separation inside the capillary are achieved via electro-osmotic flow (EOF), which is a stronger force than electrophoretic migration. This causes samples to migrate from the anode towards the cathode, where detection occurs at a specific wavelength (200 nm for protein). The technologist sees the results as an electrophoretic peak along with quantitative values. The CAPILLARYS software also automatically delimits curves and identifies fractions; therefore, in most cases operator editing is not required. Quantitative protein results can also be imported or entered by the operator—along with user-defined or free text comments—into one comprehensive chartable report.

"One nice thing about the imaging technology that's built into the CAPILLARYS, as well as Sebia's PHORESIS™ Imaging System that we use with the HYDRASYS, is that you can electronically overlay a normal curve over a questionable result to aid in interpretation," says Shearer. **"You can zoom in on a portion of the curve to more clearly see the subtleties. Before, all we could do was squint at the result on the gel."**

There's no mistaking the results

CompuNet's Medical Director, Daniel Hood, M.D., feels confident about the accuracy and quality of results generated by the CAPILLARYS 2. **"Before fully transitioning to the system, we conducted parallel testing on both the serum protein and immunotyping for a couple of months, and were very pleased with the results. Interpretation did take some getting used to because we were going from looking at a gel to looking at a screen, but overall it was a very positive transition."**

To facilitate interpretation, Sebia conducted half-day, one-on-one training with technologists onsite at



CAPILLARYS 2 – automated capillary electrophoresis

CompuNet. In addition, Dr. Hood created his own training session for the lab's pathologists. Finally, the technologists and pathologists met together for "case study" sessions where cases were reviewed on an overhead projector.

CompuNet is currently using the CAPILLARYS Protein 6

"For processing speed, ease of use and quality of results, you can't go wrong with this instrument."

(serum), Immunotyping and Hemoglobin assays. The Protein 6 assay allows for the separation of proteins into either five or six protein fractions, depending on the user's requirements. This gives the technologist a higher resolution of the beta fraction, allowing a better identification of a monoclonal protein. CAPILLARYS Immunotyping (IT) is a fully automated alternative to immunofixation (IF) testing. CAPILLARYS Hemoglobin

"...overall it was a very positive transition."

aids in the diagnosis of hemoglobinopathies and thalassemias.

The CAPILLARYS 2 assay menu also includes Urine Protein and Carbohydrate-deficient Transferrin (CDT), a contemporary biomarker for chronic alcohol abuse.

Now that CompuNet has a full year of history on the CAPILLARYS 2, would they choose this system again?

"For processing speed, ease of use and quality of results, you can't go wrong with this instrument," Shearer says.

Are you interested in learning more about the benefits of Sebia's fully automated capillary electrophoresis technology in your laboratory? Please visit us online:

<http://www.sebia-usa.com/products/capillarystestmenu.html>

SEBIA - Participation in important symposiums & conferences to help support and educate laboratorians and clinicians

Clinical Laboratory Management Association (CLMA)

Sebia has been a longtime supporter of the CLMA; we were there for the annual CLMA ThinkLab '10, which was held at the MGM Grand in Las Vegas, NV from May 3-6. The CLMA conference has always put a large emphasis on the future of the clinical laboratory industry and growth of laboratory professionals. CLMA, with its 4,000 clinical laboratory professionals, provides leadership and education to its members as well as enhances the image and increases the visibility of the profession.

International Society for Laboratory Hematology (ISLH)

Sebia also participated in the 2010 ISLH annual meeting that was held in Brighton, U.K. from May 10-12. During the annual meeting, Sebia hosted an educational luncheon highlighting hemoglobinopathy and thalassemia testing by capillary electrophoresis (CE) technology. International key opinion leaders presented educational sessions on the following topics related to Sebia's CE technology: Hemoglobin Separation and Identification by Dr. James Hoyer, Mayo Clinic; Prenatal Diagnosis of Severe Thalassemia Diseases by Dr. Supan Fucharoen, Khon Kaen University, Thailand; and Neonatal Screening for Hemoglobinopathies by Dr. Beatrice Gulbis, Hospital Erasme, Bruxelles, Belgium. The 2011 annual ISLH meeting will take place in New Orleans, LA and will be in conjunction with NASCOLA; this is an outstanding conference to discover new information in the field of hematology, especially hemoglobinopathy and coagulation testing.

Participate in the Adventure! Adventures in the Gamma Zone

Dr. Jim Faix, creator of a popular educational session ("Adventures in the Gamma Zone") presented at AACC annual meetings, would like to learn about your most interesting gamma zone adventures. The live workshop this year ("Monoclonal Gammopathy Testing", July 27, 2010) will be moderated by Jerry Katzmann, Ph.D. and feature case presentations by David F. Keren, M.D. But "Adventures in the Gamma Zone" will live on as a feature of the AACC's Clinical & Diagnostic Immunology division website. Please submit your interesting cases, in the form of electronic files, directly to Dr. Faix at JFaix@stanfordmed.org. Results may include serum and/or urine performed by agarose gel electrophoresis and/or capillary electrophoresis. Your adventure may be featured in an upcoming web-based session! To see an example, visit the AACC CDID website:

<http://aacc.org/members/divisions/immunology/pages/default.aspx>

Continued from pg 2 - Hemoglobinopathy & Thalassemia Detection

CAP requires the use of a second, complementary technique for abnormal hemoglobin results. CE is most complementary with acid gel electrophoresis and HPLC. By combining CE and HPLC methodologies, one can significantly reduce the number of unusual hemoglobin variants that can be confused with normal hemoglobins or common Hb variants (2).

Hemoglobin Newborn Screening (NBS) with Dried Blood Spot Samples

Sebia's newest FDA-cleared CE assay for hemoglobinopathy testing is CAPILLARYS NEONAT Hb FAST, for the screening of newborn blood samples collected on Guthrie Cards. Newborn dried blood spot samples are screened for the presence of normal hemoglobins (F and A) and common hemoglobin variants to include S, C, D, E, and Bart's. The system is fully automated and fast, with an instrument throughput of 96 results in 2 hours. The fast throughput is accomplished due to 8 simultaneous analyses taking place; a high-resolution 7-minute migration occurs for each newborn sample with results similar to IEF separation. Result interpretation is aided by automatically color-coded curves (normal or abnormal results) and on-board hemoglobin library by zone. All normal hemoglobins and common variants migrate in different zones - Bart's, A, F, D, S, E, A2, and C.



Aigars Brants, Ph.D.

For more information about Sebia's new Hemoglobin NBS assay for use with dried blood spots, contact us at Marketing@sebia-usa.com.

Cap Piercing CE with Whole Blood Samples

Cap piercing CE technology for hemoglobinopathy testing will soon debut with the CAPILLARYS 2 FLEX Piercing instrument*. This system has hemolyzing solution on board and will provide cap piercing, whole blood processing capabilities. The sample throughput will also be increased (37 Sample/hour) due to system design improvements. CAPILLARYS 2

Newborn Screening and Genetic Testing Symposium (NBSGTS)

The 2010 NBSGTS, sponsored by the Association of Public Health Laboratories (APHL) and the Centers for Disease Control and Prevention (CDC), took place in Orlando, FL from May 3-6. This exceptional symposium devotes four days to education sessions on topics that most affect public health laboratories. In some way, the work of public health laboratories affects the lives of all Americans. In fact, public health labs screen 97% of babies born in the U.S. for potentially life-threatening metabolic and genetic disorders, including hemoglobin newborn screening (NBS) for hemoglobinopathies. During the NBSGTS, Sebia was present as an exhibitor with the newly FDA-cleared (March 2010) CAPILLARYS™ 2 NEONAT FAST system, for the detection of normal and abnormal hemoglobins from newborn dried blood spots. This new system marks the first FDA cleared CE methodology for hemoglobin NBS.

Thalassemia International Federation (TIF)

Sebia was proud to be a Gold Sponsor for the 2010 TIF meeting held in Berlin, Germany from March 13-14. The TIF is an international organization to promote awareness about thalassemia, its prevention, medical and other care and has worked with the World Health Organization (WHO) for the past 14 years. Hemoglobinopathies are the most common, autosomal recessive disorder worldwide. Approximately 7% of the world's population is a healthy carrier of a hemoglobin disorder and 300,000 children are born annually with severe hemoglobinopathies. Prevention programs have been implemented in various parts of the world in order to diagnose carriers in childhood, before marriage, in early pregnancy, or at birth. In fact, in the U.S., every state performs hemoglobin screening on their newborn population. The Sebia CAPILLARYS 2 NEONAT FAST system will contribute to the global screening of newborn babies for hemoglobinopathies and thalassemias.

About Jim Faix, MD: Jim Faix is Director of Clinical Chemistry and Immunology at Stanford University Medical Center and Associate Professor of Pathology at Stanford University (Stanford, CA). In 2006, the AACC presented Dr. Faix with the Outstanding Speaker award for his educational session - Adventures in the Gamma Zone. Dr. Faix is currently serving as a member of the 2010 AACC Program Coordinating Committee.



FLEX Piercing will also be able to run all other assays currently available on the CAPILLARYS 2 system.

* Instrument is not yet available in the U.S.

References:

1. Szuberski J. and Hoyer J. 2009. A detailed Study of the Retention Time of Hemoglobin Variants by High Performance Liquid Chromatography (HPLC). *Int. Jnl. Lab Hem (Supplement)* 31: p.28
2. Wendt P., Durmick D., Swanson K., Herrick J., Hoyer J. 2009. Comparison of Systems Used for Identification of Hemoglobin Variants Including the Primus Ultra2 High Performance Liquid Chromatography (HPLC) System And The Sebia CAPILLARYS 2 Capillary Electrophoresis (CE) Systems. *Int. Jnl. Lab Hem (Supplement)* 31: p.29
3. Van Delft P., Lenters E., Bakker-Verweij M., De Korte M., Baylan U., Hartveld C. L., Giordano P.C. 2009. Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations. *Int. Jnl. Lab Hem* 31: 484-495
4. Keren D.F., Hedstrom D., Gulbranson R., Ou C., Bak R. 2008. Comparison of Sebia CAPILLARYS Capillary Electrophoresis with the Primus High-Pressure Liquid Chromatography in the Evaluation of Hemoglobinopathies. *Am J Clin Pathol* 130:824-831
5. Cotton F., Malaviole X., Vertongen F., and Gulbis B. 2009. Evaluation of an automated capillary electrophoresis system in the screening for hemoglobinopathies. *Clin. Lab.* 55: 217-221
6. Mais D.D., Gulbranson R.D., Keren D.F. 2009. The Range of Hemoglobin A2 in Hemoglobin Heterozygotes as Determined by Capillary Electrophoresis. *Am J Clin Pathol* 132:34-38
7. Winichagoon P., Svasti S., Fucharoen S., et al. Rapid diagnosis thalassemias and other hemoglobinopathies by capillary electrophoresis system. *Translational Research* 152 (4): 178-184
8. Hoyer J.D. 2008. Reference laboratory testing for hemoglobinopathies. *Int. Jnl. Lab Hem (Supplement)* 30: p.41