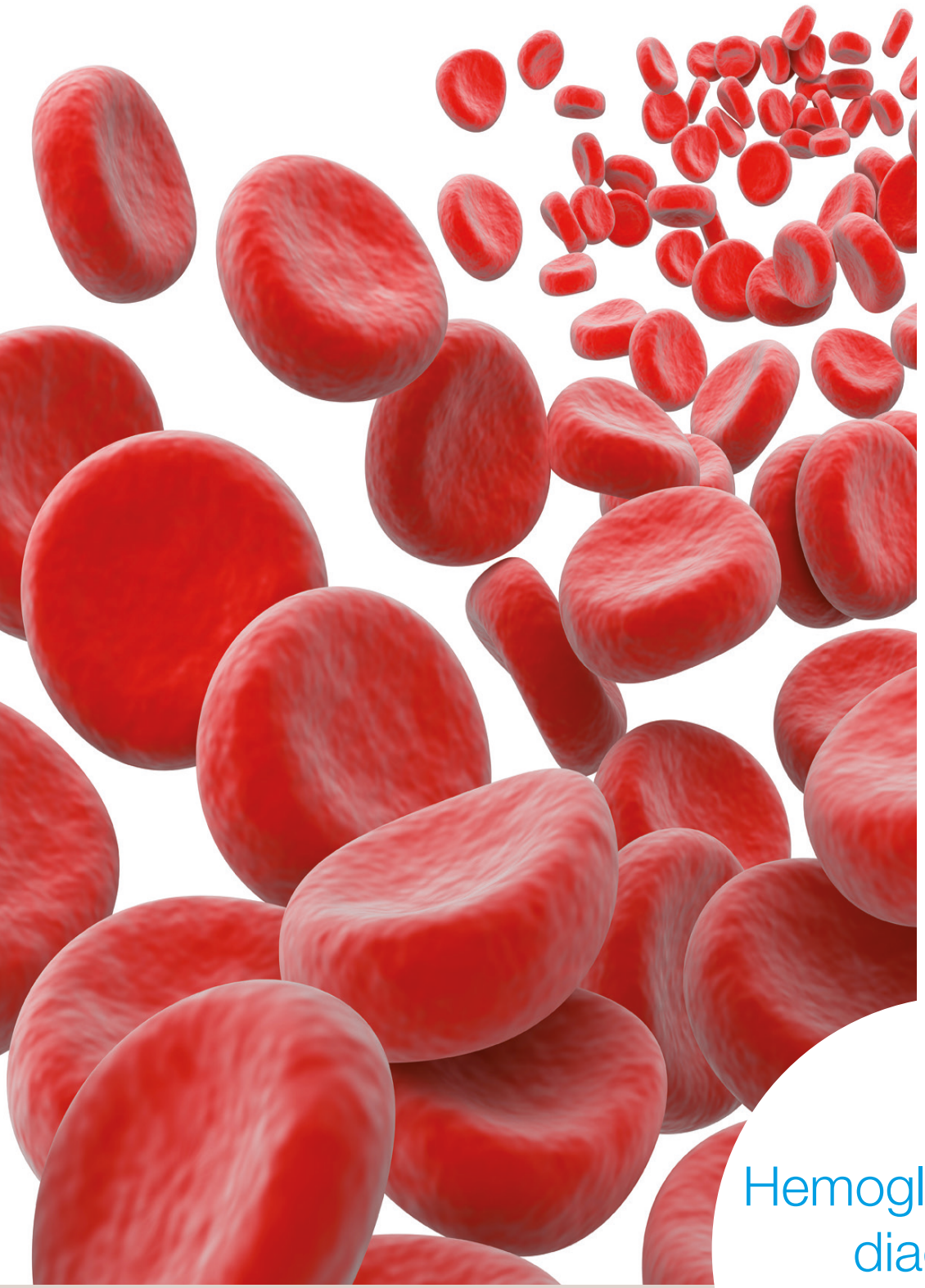


Hemoglobin

Hemoglobinopathy diagnosis by Electrophoresis

sebia

Hemoglobinopathies and Thalassemias
A global health issue

Facts and figures

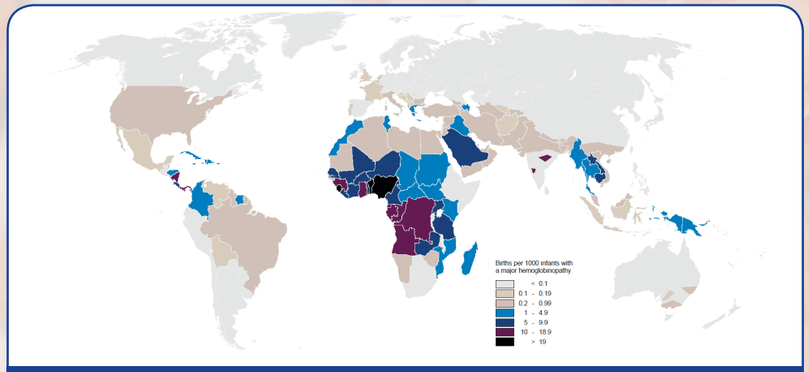
- Approximately 7 % of the global population carries some hemoglobin disorder
- According to WHO, 2.7 ‰ of conceptions are affected by severe hemoglobinopathies
- According to WHO, UNESCO and UN, Sickle Cell Disease ranks 4th in their priorities for global public health

Abnormal hemoglobins

- More than 1600 hemoglobin variants and thalassemias have been identified
- Hb S, Hb C, Hb D and Hb E, Alpha and Beta-thalassemias are the most common hemoglobin disorders worldwide

Clinical presentation

- Wide variety of phenotypes are found:
  - From asymptomatic to mild and severe clinical signs (cyanosis, anemia, sickle cell pain crisis, *in utero* death…)
  - With progressive end-organ damages (spleen, kidney, lung, retina...)



Births with a pathological hemoglobin disorder (per 1,000 live births)
(Adapted from Control of hereditary diseases: report of a WHO Scientific Group. WHO technical report series; 865 (1996)

Hemoglobin disorders are endemic in Southern Europe, Africa, Middle East and Asia. Due to the population migration, **hemoglobin disorders are common worldwide today.**

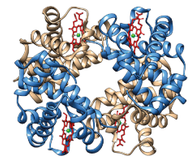
Hemoglobin disorders are **inherited genetic diseases** that affect hemoglobin:

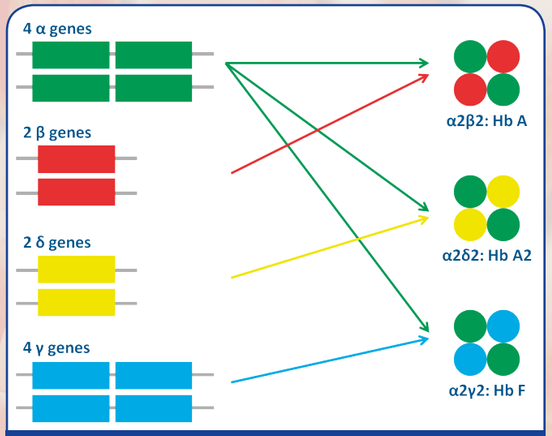
- **Qualitatively** (mutations of the globin genes impacting the structure of the hemoglobin),
  - these are called **hemoglobinopathies or Hb variants**
- **Quantitatively** (mutations or deletions of the globin genes impacting the synthesis of the hemoglobin),
  - these are called **thalassemias**
- Hemoglobin variant or thalassemia type is defined by the globin gene impacted:
  - Alpha, Beta, Delta, Gamma

Diagnosis of Hemoglobin (Hb) variants and thalassemias is crucial:

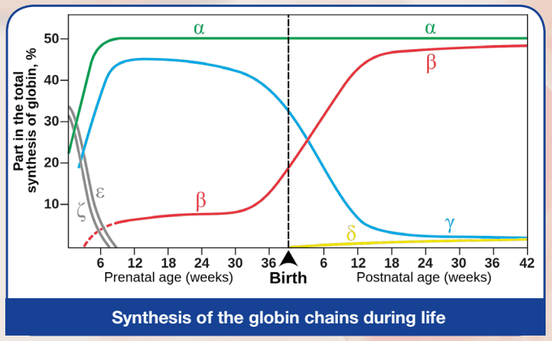
- to provide appropriate treatments and improve the quality of life in patients by preventing complications and sequela
- to offer genetic counseling in order to decrease morbidity and mortality and to reduce the financial costs associated with this condition

Physiological hemoglobins
Structure and mechanisms

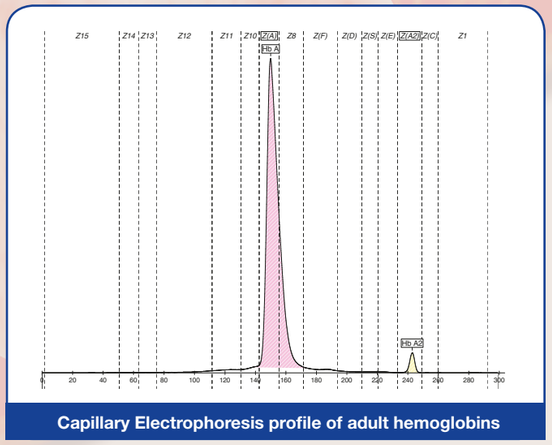




Structure of the physiological hemoglobins



Synthesis of the globin chains during life



Capillary Electrophoresis profile of adult hemoglobins

Assembly of the globin chains

- Hemoglobin consists of a tetrameric globin complex binding four Heme groups
- The type of hemoglobin is characterized by its globin chains
- The three physiological hemoglobin fractions are:
  - **Hb A** (α2β2)
  - **Hb A2** (α2δ2)
  - **Hb F** (α2γ2)

Synthesis of the globin chains

- The expression of globin chains varies during life:
  - **Gamma chains** are produced at high levels before birth but quickly decrease after birth
  - **Beta chains** quickly increases after birth
  - **Alpha chains** synthesis remain stable over life

Hemoglobin separation and quantification by capillary electrophoresis

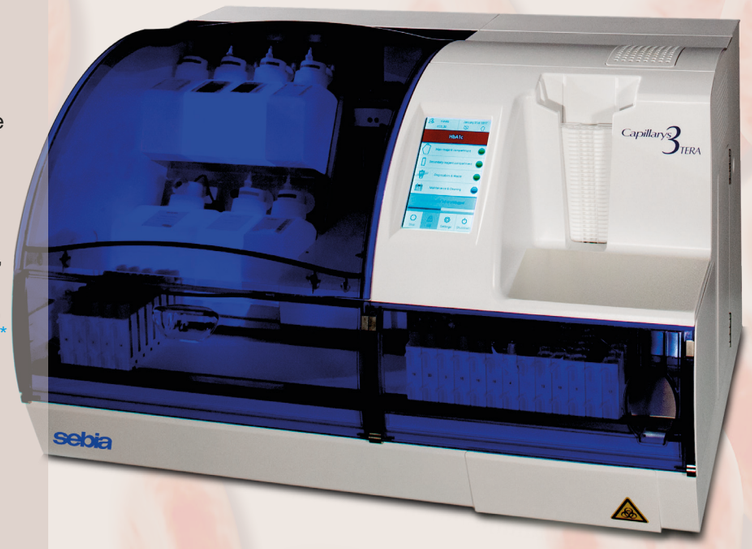
- **In adult**, the major hemoglobin fraction is Hb A.
  Hb A2 represents a minor fraction while only trace of Hb F can be found
- **In newborn**, the major hemoglobin fraction is Hb F.
  Hb A represents a lower fraction while Hb A2 is usually not yet detectable

Capillary & agarose gel electrophoresis instruments

The best-in-class technology for initial screening

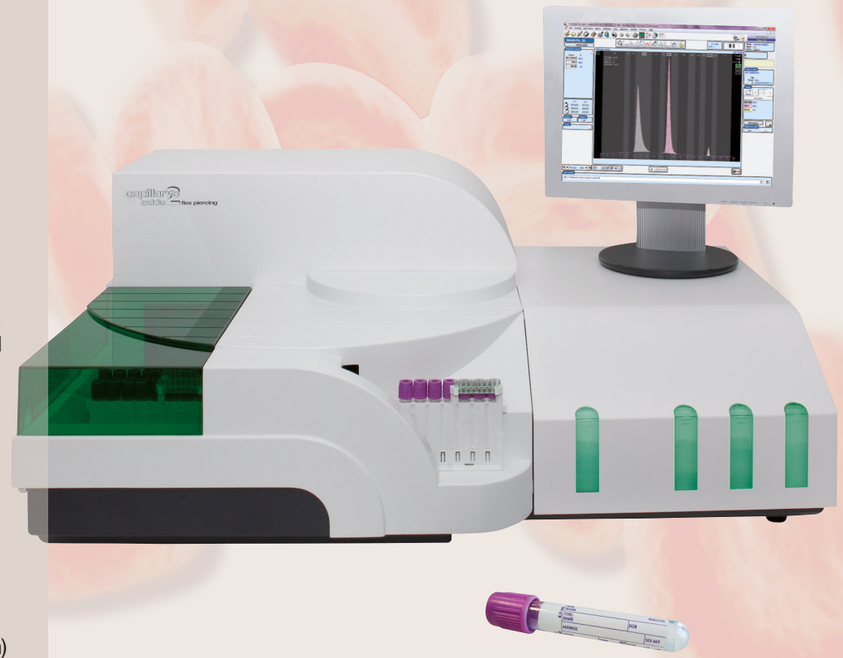
CAPILLARYS 3 TERA

- **Multi-parameter instrument**
  - Analysis on serum, urine, whole blood, capillary blood, dried blood spots (Guthrie)
- **Large test menu***
  - Protein, Immunotyping, Urine**, Urine immunotyping**, Hb A1c (venous blood, capillary blood), Hemoglobin, CDT and CDT_{IFCC} **
- **Full automation** with sample mixing and cap piercing capabilities on Hb A1c & Hemoglobin technics
- **Full traceability** from primary tube to final result
- **High throughput/12 capillaries**
  - 62 tests/h (Hb A1c & Hemoglobin)



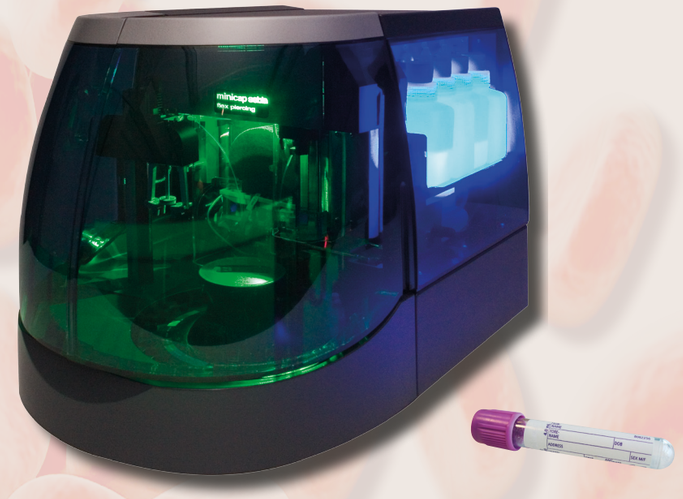
CAPILLARYS 2 Flex Piercing

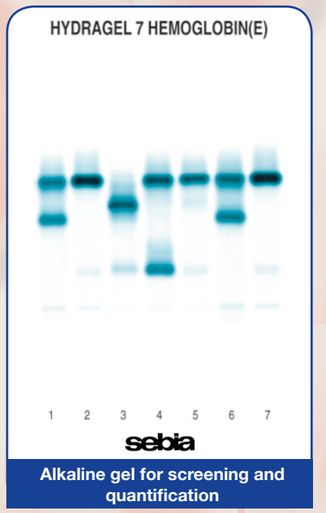
- **Multi-parameter instrument**
  - Serum, Urine, whole blood capped tube, cord blood and micro blood capillary tubes
- **Large test menu***
  - Protein, Immunotyping, Urine, Urine immunotyping, Hb A1c (venous blood, capillary blood), Hemoglobin (whole blood, cord blood), CDT and CDT_{IFCC}
- **Full automation** with sample mixing and cap piercing capabilities on Hb A1c & Hemoglobin technics
- **Full traceability** from primary tubes to final results
- **High throughput/8 capillaries**
  - 38 tests/h (Hb A1c & Hemoglobin)



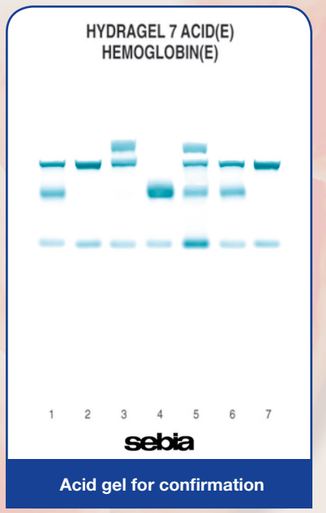
** Check with your Sebia representative for products availability*

*** Under development*





Alkaline gel for screening and quantification



Acid gel for confirmation

MINICAP Flex Piercing

- **Compact instrument**
  - Small footprint
- **Multi-parameter instrument**
  - Serum & Urine Protein
- **Large test menu***
  - Protein, Immunotyping, Hb A1c, Hemoglobin, CDT and CDT_{IFCC}
- **Full automation** with sample mixing and cap piercing capabilities on Hb A1c & Hemoglobin technics
- **Full traceability** from primary tubes to final results
- **Throughput/2 capillaries**
  - 8 tests/h (Hb A1c & Hemoglobin)

HYDRASYS 2 Scan

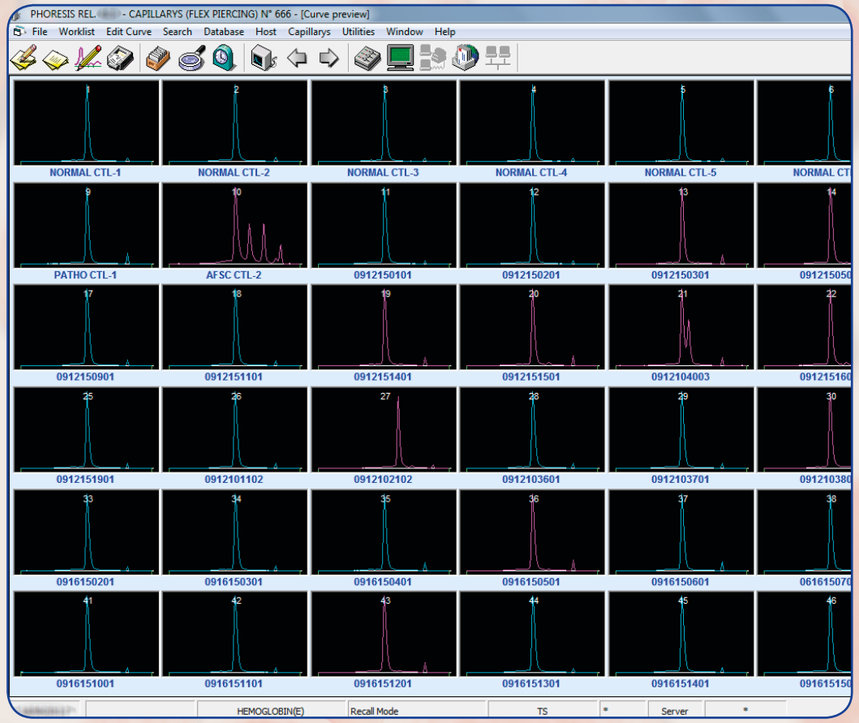
- **Compact instrument**
  - Small footprint
- **Multi-parameter instrument**
  - Washed packed red blood cells, serum, urine, CSF
- **Large test menu***
  - Hemoglobin (alkaline and acid gels), Proteins, Immunofixation, Iso-Enzymes, β2 transferrin, LDL/HDL Cholesterol, Lipoproteins
  - Isofocusing: CSF, A1AT
  - Urines: HR, Urine Profile, Bence-Jonces, Proteinuria
  - Von Willebrand factor multimers
- **Gel reading**
  - Integrated GELSCAN with high resolution CCD sensor for densitometry

Phoresis CORE validation software

Fast and easy interpretation

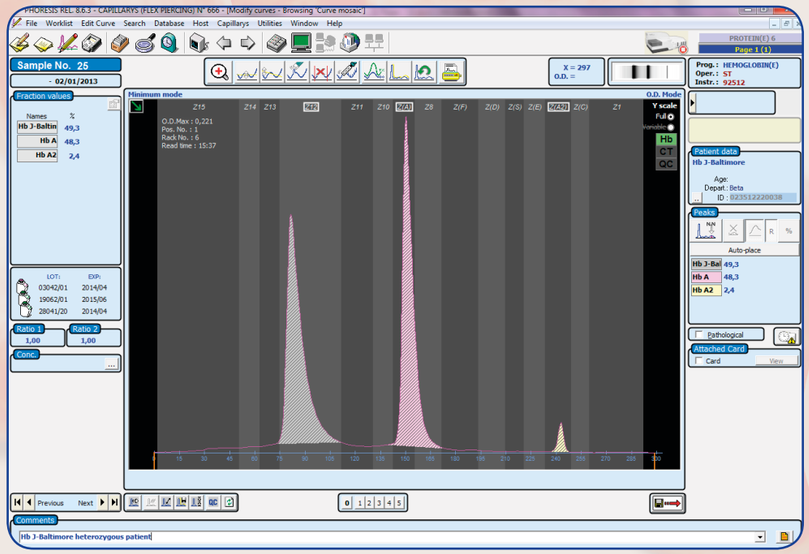
Rapid validation using Mosaic screen

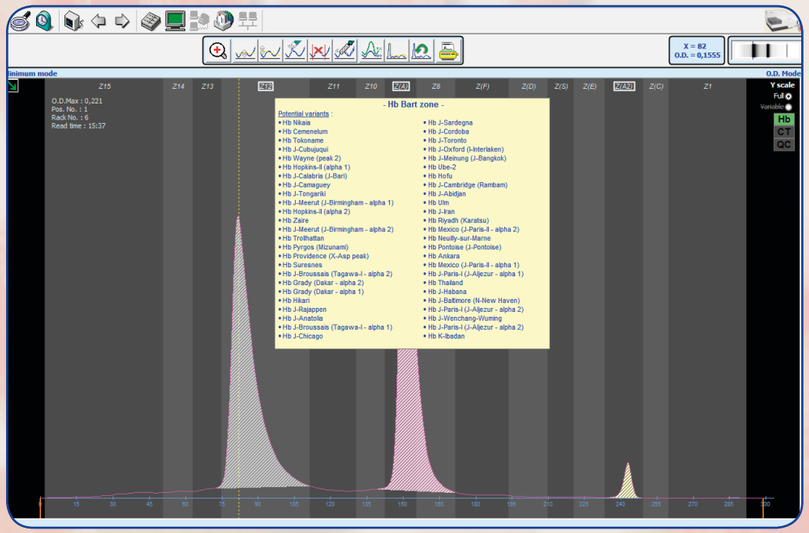
- Simultaneous visualization of:
  - 48 profiles for CAPILLARYS 2 Flex Piercing and MINICAP Flex Piercing
  - 96 profiles for CAPILLARYS 3 TERA
- Color-coded curves to sort the normal profiles from the abnormal profiles
- Advanced information program allowing to secure the results' interpretation

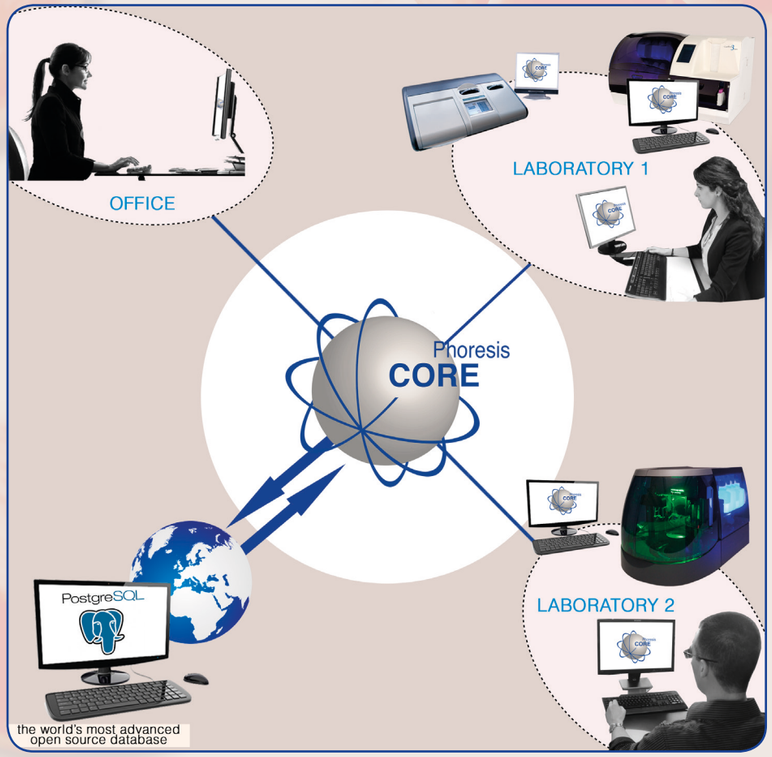


Easy interpretation through clear-cut and precise profiles

- High resolution separation of major hemoglobin variants
- Color-coded hemoglobin fractions
- Accurate quantification of hemoglobin fractions
- Intuitive and user-friendly interface
- Automatic recall of patient's history







On-board identification assistance

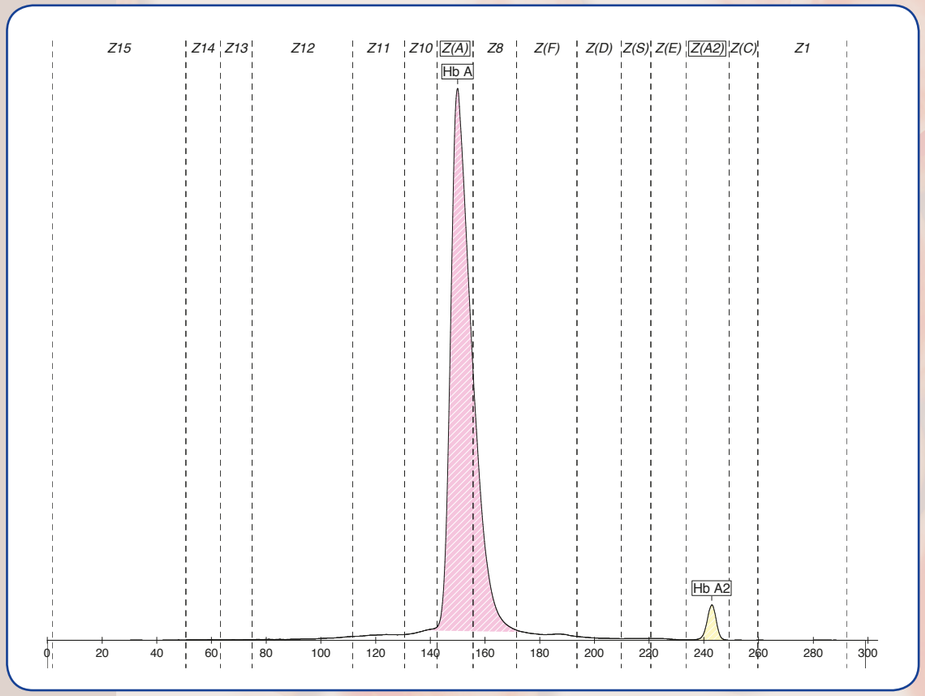
- Presumptive identification of the main hemoglobin variants by migration zones
- Drop-down library of variants migrating in each zone
- Online library of more than 360 hemoglobin variants

SQL Client/Server database

- Networking of all Sebia instruments
  - within a laboratory
  - between off-site locations
- Remote validation of the results
- Unlimited data storage
- Web access

Sebia hemoglobin capillary electrophoresis

The most comprehensive method for initial screening



- **Easy detection of the most common hemoglobin variants**
  - Full separation of Hb S, C, D and E from Hb A, A2 and F
- **Reliable screening of β-thalassemias**
  - Accurate quantification of Hb A2 and Hb F, with no interfence from Hb adducts
- **Straightforward detection of α-thalassemias**
  - Clear separation and quantification of Hb H and Hb Bart's
- **Fully automated multi-parameter instruments***
  - Hb on adult and newborn, Hb A1c, serum and urine proteins, CDT
- **High resolution at a high throughput**
  - Up to 62 tests per hour (CAPILLARYS 3 TERA)
- **Fast validation process using Mosaic screen**
  - Automatic presumptive identification and quantification

* *Check with your Sebia representative for products availability*

International Guidelines

Guidelines for the management of transfusion dependent thalassaemia (TDT).
Cappellini M.D., Cohen A., Porter J., *et al*, editors.
Nicosia, Cyprus, Thalassaemia International Federation, 2014, 3rd edition, 253 p. ISBN 978-9963-717-06-4

> ***β-thalassemia***
> *[...]*
> ***Qualitative and quantitative haemoglobin analysis***
> *Cellulose acetate electrophoresis or capillary electrophoresis (CE) and DE-52 microchromatography or high pressure liquid chromatography (HPLC) identify the amount and type of haemoglobin present.*

> ***α-thalassemia***
> *[...]*
> ***Qualitative and quantitative haemoglobin analysis***
> *Identification of fast moving haemoglobin species by electrophoresis representing HbH (β4) and Bart's (β4) is characteristic of α-thalassaemia syndromes.*

Hemoglobinopathies in pregnancy.
American College of Obstetricians and Gynecologists.
Obstetrics and Gynecology, 2007 Jan; 109(1) : 229–37

> ***Summary of Recommendations and Conclusions***
> *The following recommendations are based on good and consistent scientific evidence (Level A): [...] A complete blood count and hemoglobin electrophoresis are appropriate laboratory tests for screening for hemoglobinopathies.*

EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies.
Traeger-Synodinos J., Harteveld C.L., Old J.M., *et al.*
European Journal of Human Genetics, 2015 Apr; 23(4):426-37

> ***Haemoglobin (Hb) pattern analysis***
> *For a presumptive identification of abnormal haemoglobins at least two methods should be used. These methods include haemoglobin electrophoresis at pH 8.6 using cellulose acetate membrane, haemoglobin electrophoresis at pH 6.0 using acid agarose or citrate agar gel, isoelectric focusing (IEF), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE).*

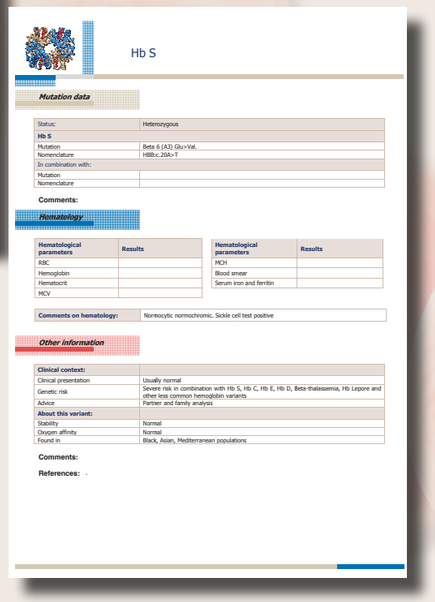
- **Indication for hemoglobin testing by electrophoresis**
  - Microcytic hypochromic anemia not caused by iron deficiency
  - Chronic hemolytic or drug-induced anemias
  - Erythrocytosis and/or cyanosis caused by hematological factors
  - Hydrops fetalis of unclear etiology
  - Prevention (testing of family members, diagnosis of partners for genetic counseling)
  - Prenatal diagnosis
- **Diagnosis and monitoring are based on:**
  - Presence of abnormal hemoglobin fractions on the electrophoresis profiles
  - Quantification of hemoglobin fractions (Hb A2, Hb F, …)

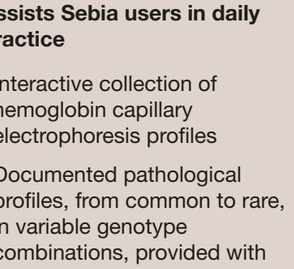
SEBIA Educational Tools*

Increasing your experience and knowledge... ... in the diagnosis of hemoglobinopathies

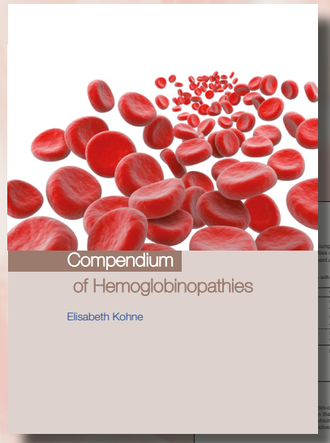
Hemoglobins ATLAS

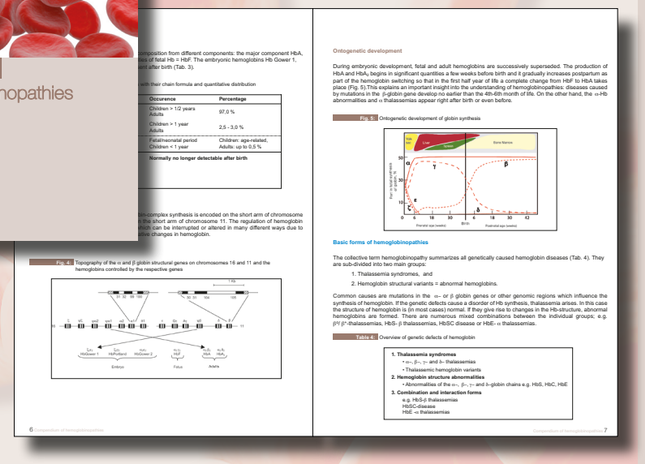
- **Assists Sebia users in daily practice**
  - Interactive collection of hemoglobin capillary electrophoresis profiles
  - Documented pathological profiles, from common to rare, in variable genotype combinations, provided with basic information on hematology and presumed risk information
  - Valuable companion in deciding when and how to confirm initial findings
- **Easy-to-use application**
  - Search and Sort functions
  - Printable reports
- **Introduction by:**
  - Pr. Piero Giordano, Emeritus Associated Professor at Hemoglobinopathies Laboratory, Leiden University Medical Center, NL

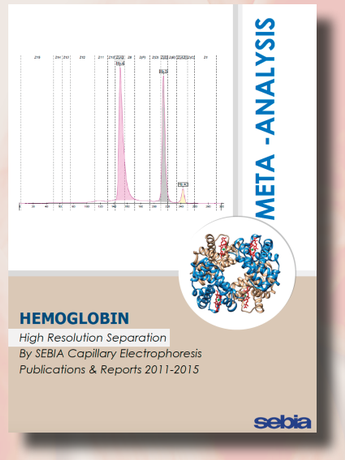


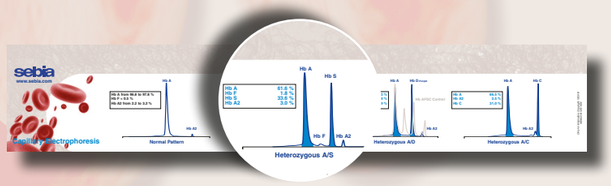


* *Available upon request from your Sebia representative*









Compendium of Hemoglobinopathies

- Educational booklet presenting hemoglobin disorders and thalassemias (laboratory diagnosis, physiopathologies, clinical presentations and treatments)
- Available in German, English, Spanish, French**
- Author: Pr. Dr. Elisabeth Kohne, Academic Head of the Hemoglobin Laboratory at the Ulm University Medical Center, Germany

Meta-analysis**

- Bibliographical review of scientific publications related to Hb testing by capillary electrophoresis

Hemoglobin ruler

- Quick reference guide to help in the interpretation of profiles

** ***Under development***

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