IVD

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free Hemoglobin (fHb)

Cyanohemiglobin Method
2 Wavelength Method (540/680 nm) acc. to Tapernon

Intended Purpose

The product "free haemoglobin (fHb) - Tapernon" is used for the spectrophotometric determination of free haemoglobin in blood (plasma) or in red cell concentrate (supernatant) with the cyanhaemiglobin method, 2-wavelength method according to Tapernon. The reagent is suitable for all 540/680 nm photometers.

Principle

Free hemoglobin found in blood plasma results from e. g. hemolytic anemia, especially hemolytic transfusion reactions.

When assessing the quality of erythrocyte or red cell concentrates (EC/RCCs), free hemoglobin is a parameter used to calculate the hemolysis rate in the RCCs.

The hemoglobin reagent quantitatively converts hemoglobin derivatives (except verdoglobin) contained in plasma or RCC supernatant into hemoglobin cyanide. This reaction is completed within 3 minutes. The formed dye (hemoglobin cyanide) is very stable and can be measured in a photometer.

To compensate turbidity (e. g. from residual erythrocyte membranes), the dual wavelength method is recommended.

Reagents

The reagent is ready for use and stable at +15 ... +25 °C until the stated expiry date. Always keep the bottle well closed and free of contamination after opening. Store the reagent protected from frost as well as direct light (sun, UV neon light).

Risks and Safety

Please observe the necessary precautions for use of laboratory reagents and body fluids. Applications should be performed by expert personnel only. Follow the national and laboratory internal guidelines for work safety and infection control. Wear suitable protective clothing and disposable gloves while handling.

It is important to ensure effective protection against infection according to laboratory guidelines.

SDS ID International
www.sds-id.com

For additional safety information please refer to the information on the label and the corresponding Safety Data Sheet (SDS).

Download by QR-Code or link: www.sds-id.com/100033-5

Contents / Main Components

004001-0250	**250 mL**	**free Hemoglobin Reagent (fHb)**
		Scattering free special quality for free Hemoglobin.
	Cont.	0.8 mmol/L cyanide, 0.61 mmol/L potassium hexacyanoferrate(III), phosphate buffer pH = 7.40, detergent / lysis reagent / stabilizer, scattering free.

Additionally required or recommended materials

004631-...		**Cyanhemiglobin-Standard fHb**
	CAL	12.0 mg/dl fHb = 1.86 µmol/l fHb.
004631-0002/5	5× 2.0 mL	Cyanhämiglobin-Standard fHb.
004631-0005/20	20× 5.0 mL	Cyanhämiglobin Standard fHb.

Sample Material

Heparinized plasma, supernatant from red cell concentrates (RCCs).

CPD-A Blood (RUO) *5).

Serum only in exceptional case of urgency.

Hemolysis caused by blood sampling and ensuing processing results in falsely elevated results.

The samples must be absolutely free of cells or other particles / fluffs (see also Performance ⇨ Interference).

Preanalytics

For preanalytics rapid sequence of steps and carefully clean work is urgently needed. Consequently, the following pre-analytical conditions generally apply to the free hemoglobin determinations.

Absolutely obsolete:

Any transportation of whole blood samples! Blood samples also cannot transported or stored when cooled. The plasma or serum must be separated immediately and carefully from the cells (pipette, not decant!).

Heparin Plasma:

Mix heparin collection tubes not too strong. Tilt 2× is usually sufficient. Then IMMEDIATELY (!!) centrifuge gently in a free-swinging centrifuge (reduced acc- and deceleration of the centrifuge). Pipette off the supernatant immediately. While holding at least 5 mm with the pipette tip to the blood cells. Don't whirl up cells! If after a repeated sharp centrifugation of the supernatant there is a sediment of cells, then it was worked insufficient neatly. Then pipette off the second supernatant again. The cell free supernatant (plasma) is used for analysis. Durability in the supernatant for several hours (under sterile conditions).

Serum *3):

Serum samples are not recommended or only for exceptional cases, because it is much more susceptible to pre-analytical hemolysis! After collection blood samples must IMMEDIATELY centrifuged and the supernatant separated (see heparin plasma). Thereafter wait until the coagulation is complete.

The supernatant is then centrifuged sharp. The cell-free serum is then removed by pipetting. First from this serum is determined the fHb *3).

RCCs:

For centrifugation follow the instructions of Plasma.

CPD-A *5):

CPD-A blood (research use only (RUO) - do not use as IVD). Keep mixing ratio exactly and consider dilution in the calculation.

Reference Ranges

The following reference values refer to the literature. Own reference values have not been determined. Variously up to 5× higher reference values are also been read. May be, very high reference values are also due to inadequate consideration of the pre-analysis.

Heparinized plasma:

< 2 mg/dl (< 20 mg/L) free hemoglobin [6].

Serum:

< 5 mg/dl (< 50 mg/L) free hemoglobin [6].

RCC Supernatant

The hemolysis rate at the end of the RCC's shelf life must not exceed 0.8 % of the erythrocyte mass *2). Depending on the respective hematocrit, this corresponds to a free hemoglobin concentration of approx. 400 mg/dl [6].

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Procedure

Wavelengths: ..... 540 nm, 680 nm
Optical path length:..... 10 mm
Temperature: ..... 20...37 °C
Measurement: ..... against reagent

Dilution 1 : 5

Pipet into tube/cuvette::		Macro:	Semi-micro:	Micro:
R	**fHb-Reagent**	**4000 µl**	**1000 µl**	**400 µl**
SA	**Sample**	**1000 µl**	**250 µl**	**100 µl**

Flush pipette tip thoroughly by repeatedly filling with reaction mixture. Mix, wait at least 3 minutes, then determine the extinction of the sample against fHb reagent as blank reagent *4).

Analysis / Calculation

At a 1 : 5 dilution calculate as follows:

Hemoglobin concetration:

$(E_{540} - E_{680}) \times 732.5$ = mg/dl fHb

$(E_{540} - E_{680}) \times 7325$ = mg/l fHb

$(E_{540} - E_{680}) \times 113.6$ = µmol/l fHb

$(E_{540} - E_{680}) \times 454.2$ = µmol/l $fHb_{(Fe)}$

Conversion:

mg/dl fHb × 0.155 = µmol/l fHb

mg/dl fHb × 0.621 = µmol/l $fHb_{(Fe)}$

Nomenclature

R = Reagent
SA = Sample
E_{000} = Extinktion/Absorption at wavelength

fHb = Tetrameric form of free haemoglobin
$fHb_{(Fe)}$ = Monomeric form of free haemoglobin

Quality Control

The use of a free hemoglobin (fHb) control is recommended to check precision and accuracy.

Examples:

- Clinchek® fHb.
  Lyophilised fHb control from Recipe www.recipe.de.
- Rapitrol® fHb.
  Liquid, ready-to-use fHb control. This control is only available as OEM production.

We recommend to use our Cyanhemiglobin Standard fHb REF 004631- in addition to a control, especially for serial measurements. Volatilities and imprecisions in the measurement can be detected much easier.

Performance

Range/Limit

This protocol is suitable to determine free hemoglobin concentrations from 0…1000 mg/dL (0…620 µmol/L) as extinction in this concentration range adheres to the Lambert-Beer law.

Precision

Intra-assay n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	149.8	0.35	0.23
Sample 2	286.6	1.11	0.39
Inter-assay n = 20	**Mean [mg/dl]**	**SD [mg/dl]**	**CV [%]**
Sample 1	128.4	0.70	0.54
Sample 2	337.0	1.34	0.40

Correlation

When comparing this reagent (y) to another reagent used in the reference method *1) (x), the following result was obtained with n = 50 samples: y = 0.9933 × + 1.7119; r = 0.996.

Interferences

We have no information about more than the interferences listed here.

Lipemia

Lipemic samples may result in increased values. Highly lipemic samples need to be clarified (ideal because without dilution effect: **Lipidex** of Bioanalytic).

Bilirubin

Icteric samples may interfere with determination of fHb. However, we have no reliable quantitative results or limits.

An interference by Bilirubin > 2 mg/dL is stated in the literature [9].

Particles

Interference can be caused by particles (dust, fluff) or cells. The 2 - wavelength method prevents true within certain limits erroneous measurements due to slight turbidity, but contrary to some interpretation it is NOT protective against erroneous measurements due to particles, lint and cells. On the contrary, the risk of erroneous measurements increased in a contaminated by particles / cell fluff with each additional wavelength or measurement.

To detect interference from particles, it is recommended to perform multiple measurements of a sample and test for compliance. Turbidity generate contrary to particles usually no differences of the same sample.

Particles from air pollution

Protect the Reagent against contamination with dust and lint and kept well closed. Ideally take the reagent by pouring in a dust and particle-free vial (e. g. Hematology cell counter vial) or rinse it before use with reagent.

At reagent remains in the bottle of less than 20 % of the stated capacity this should no longer be used for a new series (use new bottle, discard rest).

Particles from sample

To prevent particles in the sample, do not let this be open, but separate immediately after centrifugation and close.

Notes

This product information exclusively relates to the product described in this leaflet. In particular, this product information cannot be applied to similar reagents from other manufacturers.

Instruction for Use

For professional use only.

To avoid errors, the use of qualified personnel is carried out. National guidelines for work safety and quality assurance must be followed.

The used equipment must comply with the state of technology and the laboratory requirements.

Protection against infection

It is important to ensure effective protection against infection according to laboratory guidelines.

Laboratory personnel working with human samples should at a minimum be immunized against Hepatitis B (HBV).

Classifications

EU: EDMA: 13 01 09 90 00; IVD (in vitro diagnostic medical device).
AU: Class I; IVD.
CA: HC: Class I; exempt; for in-vitro diagnostic use.
US: FDA: JCG; Class I; exempt; for in-vitro diagnostic use.

Support / Information service

For methodological and technical support, please contact us by E-Mail at support@bioanalytic.de (German, English).

Periodically check for updates of this product information on our website.

Feedback

Information from users can be reported to support@bioanalytic.de (German, English).

Suggestions for further developments will be considered.

If a serious incident has occurred during or as a result of use, please report it to the manufacturer and/or its authorized representative and to your national authority.

Waste Management

Please observe your national laws and regulations.

Used and expired solutions must be disposed of in accordance with your local regulations.

Inside the EU, national regulations apply that are based on the current, amended version of Council Directive 67/548/EEG on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Decontaminated packaging can disposed of as household waste or recycled, unless otherwise specified.

Unused Remains

These are usually hazardous wastes that must be recycled or disposed of. After consultation we take back such residual materials in the original container.

Literature & Footnotes

Legends for the graphic symbols and tags used follow relevant norms or are available on our internet pages.

[1] Henry, R.J.; Clinical Chemistry; Principles and Technics, S.1134. Harper and Row, New York.

[2] DIN 58931; Hämatologie - Bestimmung der Hämoglobin-Konzentration im Blut - Referenzmethode.

[3] Zander, R., Tapernon, K.; QualiTest Heft 6, Mai 2002; Georg Thieme Verlag, Stuttgart/New York.

[4] Tapernon, K., Zander, R.; Anästhesiol Intensivmed Notfallmed Schmerzther 2001; 36 Supplement 1: S45-S50, Georg Thieme Verlag Stuttgart/New York.

[5] Williams, W.J., Beutler, E., Erslev, A.J., Lichtman, M.A.; Hematology, 4. Aufl. McGraw-Hill, New York (1990: 9).

[6] Thomas, L.; Labor und Diagnose, 4. Aufl. Med. Verlagsgesellschaft Marburg (1992: 811, 597)

[7] Rick, W.; Klinische Chemie und Mikroskopie, 6. Aufl. Springer-Verlag, Berlin-Heidelberg (1972: 115)

[8] Council of Europe Publishing. Guide to the preparation, use and quality assurance of blood components. 5th ed. 1999:84

[9] Bednar, Renate: LaboratoriumsMedizin / Journal of Laboratory Medicine. Band 18, Heft 5, Seiten 196–199, ISSN (Online) 1439-0477, ISSN (Print) 0342-3026, DOI: 10.1515/labm.1994.18.5.196, September 2009.

[10] Rüdinger, M. F.; interne Dokumentation; Bioanalytic GmbH Umkirch/ Freiburg; 2005-11-02#001.

*1) Reference method = cyanohemiglobin method acc. to DIN 58931

*2) Please observe the current guidelines.

*3) Method / procedure by Manfred F. Rüdinger (Bioanalytic GmbH). The process takes less Hämolyseeinfluss by the processing.

*4) A blank reagent mixture with fHb reagent + dist. water instead of sample should be omitted. The difference dist. water to reagent is not measuring-oriented relevant and the use of dist. water carries the risk of introducing scattering particles.

*5) CPD-A blood causes shifts in the pH value and incorrect results in the case of incorrect mixing ratios (too less blood in sampling tubes), as well as measurement errors due to precipitation. Do not use CPD-A blood for IVD - only for research (RUO).