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free Hemoglobin (fHb)

Determination of Free Hemoglobin (fHb)
3 Wavelength Method (415/380/450 nm) acc. to Harboe

Intended Purpose

The product "free haemoglobin (fHb) - Harboe" is used for the spectrophotometric determination of free haemoglobin in plasma, serum or in the supernatant of erythrocyte concentrates with the 3-wavelength method according to Harboe [1]. The method can be used on all spectrophotometers and clinical-chemical analysers with adjustable wavelengths 415/380/450 nm.

Principle

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

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

As hemoglobin is measured in its native state, i.e. without chemical conversion, the hemoglobin derivatives contained in the sample are not detected by the Harboe triple wavelength method. Any relevance of this needs to be checked for individual determinations.

After sufficient mixing with the reagent, the sample can be photometrically measured right away *2).

Reagent

The reagent for the Harboe fHb method is ready for use.

At the storage temperature indicated on the label the reagent has a shelf life until the printed expiry date.

Always keep the bottle well closed and free of contamination after opening. Obtain the required amount solely by pouring. Do not use the reagent if the solution is not clear and free of particles.

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.

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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/100138-7

Contents / Main Components

004003-...		free hemoglobin (fHb) acc. to Harboe
	Cont.	Scattering free, stabilized TRIS/HCl buffer solution with physiological Osmolality of 288 mosm/kg H_2O.
004003-0020/5	5× 20 mL	free hemoglobin (fHb) acc. to Harboe
004003-0250	1× 250 mL	free hemoglobin (fHb) acc. to Harboe.

Additionally required or recommended materials

	Photometric equipment, pipettes.
005190-...	Lipidex (for clearing highly lipemic samples) *

* available from Bioanalytic

Sample Material

Heparinized plasma, serum (limited suitability), supernatant from red cell concentrates (RCCs)

Hemolysis during preanalytics (blood sampling and ensuing processing) results in falsely elevated results.

The samples must be absolutely free of cells or other particles / lint (see also: Capability Characteristics > Interferences).

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.

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 [2].

Serum:

< 5 mg/dL (< 50 mg/L) free hemoglobin [2].

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: ..... 415 / 380 / 450 nm
Optical path length: ..... 10 mm
Temperature: ..... 20...37 °C
Measurement: ..... against reagent

Dilution 1 : 11

Other dilutions can be used taking into account the linearity range and after validation.

At values above 200 mg/dL choose a higher correspondingly dilution with reagent.

At values below 5 mg/dL Harboe recommends a dilution of 1 : 6.

Pipet into tube/cuvette:		Macro:	Semi-micro:	Micro*:
SA	**Sample ****	**1.0 ml**	**500 µl**	**200 µl**
R	**Reagent**	**10.0 ml**	**5.00 ml**	**2.00 ml**

* Ein Ansatz mit geringeren Volumina ist nicht zu empfehlen.

** For the triple-wavelength method, at least triplicate measurements of samples from separate preparations are recommended (see ⇨ interferrences ⇨ particles).

Flush pipette tip properly by repeatedly filling with reaction mixture. Mix thoroughly. Measure as soon as air bubbles have risen to the top [*2)].

Analysis / Calculation

To calculate values, an Excel sheet is available for download.

For Dilution 1 : 11:

$((168 \times A_{415}) - (84 \times A_{380}) - (84 \times A_{450}))$ = mg/dL fHb
$((168 \times A_{415}) - (84 \times A_{380}) - (84 \times A_{450})) \times 10$ = mg/L fHb

For Dilution 1 : 6:

$((168 \times A_{415}) - (84 \times A_{380}) - (84 \times A_{450})) \times 0.545$ = mg/dL fHb
$((168 \times A_{415}) - (84 \times A_{380}) - (84 \times A_{450})) \times 5.45$ = mg/L fHb

Conversion:

mg/dl fHb × 0.155 = µmol/l fHb

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

Nomenclature

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

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

Quality Control

The national guidelines for quality assurance must be followed.

Suitable controls should be conducted with each application in order to avoid an incorrect result. For control of precision and accuracy using a suitable control material is recommended [*3)].

Capability Characteristics

Range/Limit

For a 1 : 11 dilution, the lower limit of the Harboe method is 5 mg/dL fHb and the upper limit is 200 mg/dL fHb.

At a dilution of 1 : 6, this corresponds mathematically to a lower limit of 2.73 mg/dL or an upper limit of 109 mg/dL.

Precision for dilution 1 : 11

In der Serie n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Samle 5.0 mg/dL	4.48	0.17	3.76
Samle 7.5 mg/dL	6.34	0.18	2.83
Samle 10 mg/dL	8.92	0.29	3.21
Samle 30 mg/dL	27.63	0.42	1.52
Samle 50 mg/dL	47.97	0.61	1.26
Samle 75 mg/dL	71.48	0.76	1.07
Samle 100 mg/dL	98.58	1.24	1.26
Samle 150 mg/dL	143.1	2.14	1.50
Samle 200 mg/dL	194.2	2.13	1.10

Correlation of dolution 1 : 11

A gravimetrically/volumetrically traceable comparison of this reagent (x) with a standard dilution series (y) was performed. A stock solution of this dilution series was measured against the reference for the hemoglobin cyanide method acc. to DIN 58931. Analyses were performed at a 1:11 dilution.

11 standard values from 0…200 mg/dL were measured. One standard value comprised 20 determinations.

A total of n=220 measurements yielded the following result: y = 1.0286 × +0.8074; r = 0.99974.

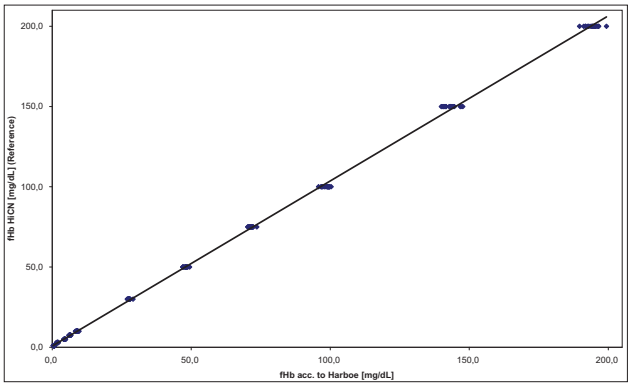

Fig. 1: Dilution 1 : 11; all results

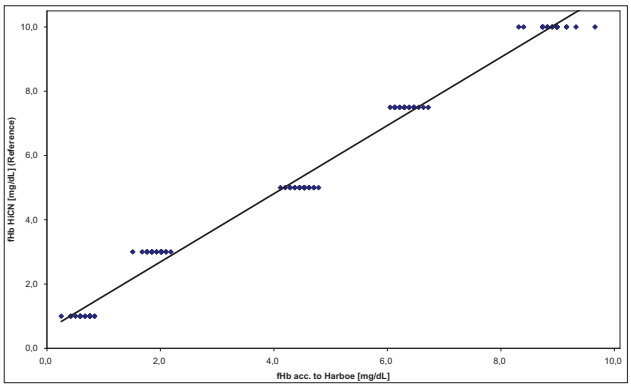

Fig. 2: Dilution 1 : 11; values below 10 mg/dL

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 Bio-analytic GmbH).

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 [4].

Particles

The high-quality Bioanalytic fHb reagent is free from scattering and is produced under particle and dust-free conditions.

Interference can be caused by particles (dust, fluff) or cells. The 3 - 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 several times 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).

Never transfer remains of a bottle into another!

Particles from sample

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

Notes

For professional use only.

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.

Classifications

EU: EDMA: 13 01 09 90 00; IVD (in vitro diagnostic medical device).

Support / Information service

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

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

Feedback

Information from users can be reported to support@bioanalytic.de.

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] M. Harboe: A Method for Determination of Hemoglobin in Plasma by Near-Ultraviolet Spectrophotometry. Scandinavian Journal of Clinical & Laboratory Investigation Jan 1959, Vol. 11, No. 1: 66–70.

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

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

[4] Bednar, Renate; Bayer, P. M.: Freies Hämoglobin im Plasma - Vergleich zweier Spektralphotometrischer Methoden - Bilirubin als Störfaktor; ZB MED Lab.med. 18: 198 (1994).

*1) Please observe current applicable laws and regulations.

*2) Depending on type and vigorousness of mixing, air bubbles can be created. Wait for air bubbles to rise to the top. This process can be gauged by control until the photometrically determined value is stable (only for constant measurements – refer to the instructions for your photometer).

*3) Bioanalytic can manufacture ready-to-use, method-independent controls. We have already developed these in 2005 after a suggestion by the Paul-Ehrlich- Institute (PEI). The typical deviation is only about ±10 % and is independent of the chosen method – i. e. identical for the hemoglobin cyanide, triple wavelength as well as any other method!
Production and minimum order quantities on request (no small-volume production for users).