IVD

METHOD COMPARISON

CE en

free Hemoglobin (fHb)

Two different methods are available for free hemoglobin.
Here you will find the main differences between the two methods.

Preanalytics

The preanalytics described in the instructions for use of the reagent is fundamentally important! If pre-analysis is ignored, incorrect determinations are pre-programmed. This applies in principle to all fHb methods - independent of method and manufacturer!

2-wavelength method (540/680 nm) according to Tapernon [1] (cyanhaemiglobin method)

Method

The cyanhaemiglobin method for haemoglobin determination is a recognized reference method described in the DIN 58931 standard. Cyan hemiglobin is a very stable compound and photometrically easy to measure. It includes all hemoglobin derivatives except verdoglobin.

The cyanhaemiglobin method was modified by Tapenon [1] for free hemoglobin as a 2-wavelength method.

Applicability

The Bioanalytic reagents designated for fHb are specially produced without scattering and tested for fHb. Scatteringless reagents are particularly required for all multi-wavelength methods. Otherwise, there is a risk of individual incorrect measurement results due to the calculation – up to negative results.

Adaptability

The method is very easy to adapt to automatic analysers. The basis for this is our manual for manual execution.

Please note that the sample volume should not be less than 100 µl. Below this sample volume, the method is outside the specification or CE conformity.

Application instructions

We do not have application instructions for automatic analysers ourselves. To do this, please contact your device manufacturer or their service department for application.

Some device manufacturers refer their device customers directly to us on request for fHb. As a rule, these device manufacturers also have application instructions or can help you.

Comparison

- Highly lipaemic samples can lead to increased or even lower results and should always be clarified with Lipidex *2) (Bioanalytic) before analysis.
- Interferences due to bilirubin are given by the measuring wavelength. However, we have no reliable quantitative results or limit values.
- Interferences caused by incoming particles from the ambient air can interfere with a correct measurement.
- Simple calculation of the fHb concentration via factor/calculation scheme.
- A cyan haemiglobin standard is available for calibration or for checking when calculating with Factor.

3-wavelength method (380/415/450 nm) according to Harboe [2]

3-wavelength method (415/450/700 nm) according to Fairbanks [3]

Method

The 3-wavelength methodes according to Harboe [2] and Fairbanks [3] are a native measurement of hemoglobin in a buffered and scattering-free solution. The detection relates exclusively to the molecule hemoglobin. Hemoglobin derivatives are not included.

Applicability

The Bioanalytic reagents designated for fHb are specially produced without scattering and tested for fHb. Scatteringless reagents are particularly required for all multi-wavelength methods. Otherwise, there is a risk of individual incorrect measurement results due to the calculation – up to negative results.

Adaptability

The method is very easy to adapt to automatic analysers. The basis for this is our manual for manual execution.

Please note that the sample volume should not be less than 200 µl. Below this sample volume, the method is outside the specification or CE conformity.

Application instructions

We do not have application instructions for automatic analysers ourselves. To do this, please contact your device manufacturer or their service department for application.

Some device manufacturers refer their device customers directly to us on request for fHb. As a rule, these device manufacturers also have application instructions or can help you.

Comparison

- Highly lipaemic samples can lead to increased or decreased (also negative) results and should always be clarified with Lipidex *2) (Bioanalytic) before analysis.
- Interferences by bilirubin are less likely due to the measuring wavelength than with the cyanhaemiglobin method. However, we have no reliable quantitative results or limit values.
- Interferences caused by incoming particles from the ambient air can interfere with a correct measurement. Here, the 3-wavelength methodes are a little more sensitive to interferences.
- Simple calculation of the fHb concentration via factor/calculation scheme.
- There is no standard for calibration available.

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- Controls available on the market [*3)] can be used for your internal quality control. Method-specific nominal values of these controls must be observed if necessary. These controls may have method-specific target values due to the manufacturing process (lyophilization).
- For the direct method comparison/traceability to HiCN standard according to CLSI, we recommend freshly prepared liquid samples as far as possible.
  There are currently no commercially available liquid controls available for this routine.
- A protocol is available on request for the in-house production of liquid controls. However, the high workload is only profitable for the validation, less for the routine. As a rule, these liquid controls show no significant difference between different analytical methods.
- Liquid controls can be custom-made by Bioanalytic. Minimum quantity is 5 litres per batch. Profitability is usually not given for users/test laboratories, since a requirement of > 2 ... 3 L per month is required.

Usage

Basically, both methods for fHb determination are largely equivalent. However, there are specific advantages for the different applications.

- Adaptable to automatic analysers.

Primary use

In vitro diagnostic (IVD)

- As the only or parallel method.

Referencing

- Use for referencing the harboe method, as a standard is available.

Material check

- The fHb determination based on the cyanhaemiglobin method is mainly used in material testing (haemolysis properties of material surfaces for medical applications), in some cases also parallel to the method according to Harboe.

Transfusion medicine

- This method is also well suited for transfusion medicine. After replacing the donor plasma with synthetic and microfiltered solution mixtures, there are almost no interferences.

- Controls available on the market [*3)] can be used for your internal quality control. Method-specific nominal values of these controls must be observed if necessary. These controls may have method-specific target values due to the manufacturing process (lyophilization).
- For the direct method comparison/traceability to HiCN standard according to CLSI, we recommend freshly prepared liquid samples as far as possible.
  There are currently no commercially available liquid controls available for this routine.
- A protocol is available on request for the in-house production of liquid controls. However, the high workload is only profitable for the validation, less for the routine. As a rule, these liquid controls show no significant difference between different analytical methods.
- Liquid controls can be custom-made by Bioanalytic. Minimum quantity is 5 litres per batch. Profitability is usually not given for users/test laboratories, since a requirement of > 2 ... 3 L per month is required.

Usage

Basically, both methods for fHb determination are largely equivalent. However, there are specific advantages for the different applications.

- Adaptable to automatic analysers.

Primary use

In vitro diagnostic (IVD)

- The fHb determination according to Harboe is mainly used in the field of in-vitro diagnostics (medical and vet.-med. laboratory).
  The fHb cyanhaemiglobin method is then used for referencing.

Literature & Footnotes

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

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

[2] 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.

[3] V.F. Fairbanks et al. Methods for measuring plasma hemoglobin in micromolar concentration compared. Clin. Chem., 38:132{140, 1992.

- All information given here is based on our experience, feedback from customers and is without guarantee.

1) See method instructions.

2) LipidEx is available from Bioanalytic GmbH. REF 005190-0010 (10 ml) or 005190-0100 (100 ml). 500 to 1000 µl are used per sample. There is no dilution factor to consider!

3) RECIPE Chemicals + Instruments GmbH • www.recipe.de • Dessauerstrasse 3 • 80992 Munich.