METHOD: Azide Methemoglobin

SPECIMEN: Whole Blood (Fingerstick or Venous)

ASSAY TEMPERATURE: Room Temperature

CONTROLS: HemoPoint® H2 Hemoglobin Tri-Level Controls (Cat. No. 3060-601) or HemoPoint® H2 Hemoglobin Bi-Level Controls 3065-601), if external controls are necessary to comply with local or other regulations

LIMITATIONS:

1. The microcuvette sample can be measured immediately or within 10 minutes at the latest, otherwise false results may be obtained

2. Air bubbles in the optical eye, caused by inadequate filling of the microcuvette cavity, may cause false results. Discard the microcuvette and take another sample using a new microcuvette.

3. Ensure that you do not hold the microcuvette at its filling end, because this may contaminate the optical eye.

4. In order to avoid contamination of the cuvette holder, remove surplus blood from the outside of the microcuvette.

5. All results above 23.5 g/dL or equivalent must be confirmed by laboratory method.

6. Sulfhemoglobin is not measured by this method. Carboxyhemoglobin and turbidity due to leukocytosis or hyperlipemia do not interfere.

PRINCIPLE:

The HemoPoint® H2 or HemoPoint® H2 DMS system is intended to be used for the quantitative determination of hemoglobin (Hgb) concentration in human blood. It consists of a photometer instrument and individual single-use microcuvettes filled with reagents. Using the microcuvette, a small amount of capillary, venous or arterial blood is taken up by capillary action. The microcuvettes are intended to be used once only and must be disposed of after use as potentially infectious waste, in accordance with the current regulations applicable to your establishment. The HemoPoint® H2 system is designed for use in medical practices and in clinical laboratories to assist in medical diagnostics. In addition it can be used in emergency and intensive care units and in medical facilities such as blood donor sessions and blood banks. Blood sampling and operating the HemoPoint® H2 system should be carried out by trained personnel with sound knowledge of the system.

The recognized reference method for total hemoglobin is the cyanmethemoglobin method, which is also known as the cyanhemoglobin method. The blood sample is diluted 1:251 with a reagent buffering solution. Here the erythrocytes are hemolyzed and the bivalent iron in oxyhemoglobin and deoxyhemoglobin are oxidized by the reagent potassium hexacyanoferrate (III) to trivalent iron and so converted to methemoglobin. Together with cyanide ions, which are also contained in the reagents, the methemoglobin forms a stable, colored complex, namely cyanmethemoglobin. This has a wide absorption maximum at 540 nm. This absorption is proportional to the Hgb concentration. In 1966, Vanzetti suggested to replace KCN by NaN₃ and thus he was able to reduce the toxicity of the reagent mixture considerably. Vanzetti’s method is also known as the azide methemoglobin method. A modified azide met-hemoglobin method is used in the HemoPoint® H2 system.
Principles of the Procedure

In the HemoPoint® H2 or HemoPoint® H2 DMS system, the use of HemoPoint® H2 n x t Microcuvettes with short light pathways makes it possible to analyze undiluted blood. The filled microcuvette is inserted into the HemoPoint® H2 or HemoPoint® H2 DMS photometer, the color produced by the chemical reaction in the cuvette is measured, and the hemoglobin level is calculated and displayed.

For this purpose, light is directed through the blood sample and the absorption is measured. From the amount of light absorbed by the sample, the concentration of the hemoglobin in the cuvette can be calculated using the Beers-Lambert Law. Light emitting diodes (LED's) are used as light sources and a photodiode is used to detect the light. The light emitting diodes utilize the central wavelengths 570 nm (for measurement) and 880 nm (for turbidity compensation).

The Microcuvette

The plastic microcuvette consists of a clear body with a cavity which takes up approximately 8 µL of blood which combines with the dry reagent chemistry. The optical distance between the cuvette walls is fixed and permits photometric determination of the hemoglobin in undiluted blood samples.

The Chemistry Principle

In order to use the azide methemoglobin method in undiluted blood, three reagents are necessary: sodium deoxycholate dissolves and disperses the cell walls of the red blood corpuscles. Hence the hemoglobin formerly contained in the erythrocytes is now available free in the solution. The bivalent iron of the oxyhemoglobin and the deoxyhemoglobin becomes oxidized by sodium nitrite NaNO₂ to trivalent iron, in methemoglobin. Existing and formed methemoglobin and azide ions from sodium azide NaN₃ form a colored complex which exhibits maximal absorption at 540 and 575 nm and hence it can be quantitatively determined photometrically.

STORAGE:

HemoPoint® H2 n x t Microcuvettes are to be stored solely in the original container and at room temperature 59 – 86 °F (15 – 30 °C). Do NOT refrigerate! Use microcuvettes within 3 months after opening container. Document the initial opening date on the container label in the space provided. Only remove one microcuvette at a time from the container and then immediately close the lid. The microcuvettes are analyzed optically in the HemoPoint® H2 or HemoPoint® H2 DMS photometer.

Measurement light must pass through the sample cuvette to the photo detector with the least possible interference. It is therefore crucial not to touch the optical eye of the cuvette with fingers, dirty or sharp objects.

SPECIMEN COLLECTION AND PREPARATION:

The HemoPoint® H2 or HemoPoint® H2 DMS Photometer can be used with capillary, venous, or arterial blood. Use EDTA, heparin or heparin/fluoride as anticoagulants, preferably in solid form, to avoid dilutional effects. Venous and arterial blood samples may be used if the blood collected is not more than 24 hours old and the samples have been stored refrigerated 35 – 46 °F (2 – 8 ºC).
Prepare stored samples for measurement as follows:
1) Remove sample tube from the refrigerator and bring it to room temperature.
2) Mix the sample tube well. (i.e. by a mechanical rotator or hand inversion at least 10 times).

PROCEDURE:

Instructions For Use (Capillary)

1. Make sure that the Photometer is ready for use. See the HemoPoint® H2 or HemoPoint® H2 DMS User’s Guide for the device.
2. Make sure that your patient is sitting comfortably.
3. There should be a good blood circulation in the hand of from which you wish to take blood, e.g., it should be warm and relaxed.
4. Lightly massage the fingers, in order to stimulate circulation.
5. Disinfect the puncture site and allow to dry.
6. Take out a microcuvette from the container and close the lid immediately.
7. Press lightly on the fingertip and puncture with a suitable sampling device on the side of the fingertip.
8. Blot away the first drop of blood then, if necessary, press gently once again to get a 2nd drop of blood which is large enough to fill the microcuvette completely. Avoid “milking” the finger.
9. Hold the tip of the microcuvette tip in the middle of the drop of blood and let the cavity fill in one step. In case of air bubbles in the optical eye, discard the microcuvette and take another sample using a new microcuvette.
10. In order to avoid contamination of the cuvette holder, remove surplus blood from the outside of the microcuvette.
11. The microcuvette sample prepared in this way can now be measured immediately or within 10 minutes at the latest.

Instructions For Use (Venous or Arterial)

1. Make sure that the Photometer is ready for use. See the HemoPoint® H2 or HemoPoint® H2 DMS User’s Guide for the device.
2. Remove sample tube from the refrigerator and bring it to room temperature.
3. Mix the sample tube well (i.e., by a mechanical rotator or mixing by hand at least 10 times).
4. Take out a microcuvette from the container and close the lid immediately.
5. Pipette a sufficient drop of blood on a non-absorbent material (i.e., plastic film).
6. Hold the tip of the microcuvette in the middle of the drop of blood and let the cavity fill in one step. In case of air bubbles in the optical eye, discard the microcuvette and take another sample using a new microcuvette.

7. In order to avoid contamination of the cuvette holder, remove surplus blood from the outside of the microcuvette.

8. The microcuvette sample prepared in this way can now be measured immediately or within 10 minutes at the latest.

Reagents

HemoPoint® H2 n x t Microcuvettes, Cat. Nos. 3015-100 (2 x 50 size canister of microcuvettes) or 3015-200 (4 x 50 size canister of microcuvettes).

Additional Materials Needed

HemoPoint® H2 or HemoPoint® H2 DMS Photometer
HemoPoint® H2 Hemoglobin Tri-level Controls (Cat. No. 3060-601) or HemoPoint® H2 Hemoglobin Bi-level Controls (Cat. No. 3065-601), if external controls are necessary to comply with local or other regulations
Disposable pipettes (for venous or arterial blood only)
Plastic film (for venous or arterial blood only)
Lint-free material

RESULT:

The test result is displayed directly on the screen of the HemoPoint® H2 or HemoPoint® H2 DMS photometer. No calculations are necessary. The test is linear up to 23.5 g/dL.

QUALITY CONTROL:

AutoCheck:

The HemoPoint® H2 or HemoPoint® H2 DMS AutoCheck performs an internal check of the photometer’s optic system every time the cuvette holder is opened.

External Quality Control:
If additional quality control is required by local or other regulations, external control material may be used. For this purpose, we recommend the use of Stanbio’s HemoPoint® H2 Hemoglobin Tri-level Controls, Cat. No. 3060-601 or HemoPoint® H2 Hemoglobin Bi-level Controls, Cat. No. 3065-601.

Do not use cyanmethemoglobin derived standards or controls with this test.

REFERENCE: HemoPoint® H2 Instruction For Use, RBR.3015 (current version)

Date of Review/Revision
Reviewed by____________________________
Lab Director/Supervisor

DN: NCCLS.3015.02 rev. 06/10