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Alere HemoPoint® H2 Microcuvettes Laboratory Procedure

This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. **Any modifications to this document are the sole responsibility of the Facility.**

CLIA Complexity: Waived

1. Intended Use

The Alere HemoPoint® H2 Microcuvettes are intended to be used in the Alere HemoPoint® H2 Meter. The reagents/ microcuvettes and the Alere HemoPoint® H2 Meter form an analytical system.

2. Summary and Principle

The Alere HemoPoint® H2 System is intended to be used for the quantitative determination of hemoglobin (Hgb) concentration in human blood. It consists of a meter instrument and individual single-use microcuvettes filled with reagents. Using the Alere HemoPoint® H2 Microcuvette, a small amount of capillary, venous or arterial blood is taken up by capillary action. The Alere HemoPoint® H2 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 Alere 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 Alere 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.¹ The blood sample is diluted 1:251 with a reagent buffering solution. Here the erythrocytes are hemolyzed and the bivalent iron in oxyhemoglobin and desoxyhemoglobin 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.² Vanzetti's method is also known as the azide methemoglobin method. A modified azide methemoglobin method is used in the Alere HemoPoint® H2 System.



3. Principles of the Procedure

In the Alere HemoPoint® H2 System, the use of Alere HemoPoint® H2 Microcuvettes with short light pathways makes it possible to analyze undiluted blood. The filled Alere HemoPoint® H2 Microcuvette is inserted into the Alere HemoPoint® H2 Meter, the color produced by the chemical reaction in the Alere HemoPoint® H2 Microcuvette 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 Alere HemoPoint® H2 Microcuvette 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).

A. The Alere HemoPoint® H2 Microcuvette

The plastic Alere HemoPoint® H2 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 Alere HemoPoint® H2 Microcuvette walls is fixed and permits photometric determination of the hemoglobin in undiluted blood samples.

B. 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 become 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.

4. Reagents

A. Materials Provided

Component	Content	Quantity
Microcuvettes Ref.No. 80549	40% w/w sodium deoxycholate, 20% sodium azide, 20% w/w sodium nitrite and 20% w/w non-reactive ingredients.	100
Package insert		1

B. Materials Required But Not Provided

- Alere HemoPoint® H2 Meter³
- Alere HemoPoint® H2 Control Cuvette (optional)
- Alere™ Hemoglobin Controls (Ref. No. 88772) (optional)
- Disposable pipettes (venous or arterial blood only)
- Plastic film (venous or arterial blood only)
- Lint-free material



5. Warnings and Precautions

Alere HemoPoint® H2 Microcuvettes are designed for in-vitro diagnostic use only. The reagents which coat the inner walls of the Alere HemoPoint® H2 Microcuvettes are harmful and must not be swallowed. Wear suitable protection (gloves) at all times when handling blood samples. Please note that all human blood samples or products must be handled as potentially infectious waste per your local regulations.

6. Storage and Handling

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

Measurement light must pass through the sample Alere HemoPoint® H2 Microcuvette to the photo detector with the least possible interference. It is therefore crucial not to touch the optical eye of the Alere HemoPoint® H2 Microcuvette with fingers, dirty or sharp objects.

7. Specimen Collection and Preparation

Specimen	The Alere HemoPoint® H2 Meter 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°C).
Specimen Storage	Prepare stored samples for measurement as follows: <ol style="list-style-type: none"> 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).

8. Procedure

Refer to the Alere HemoPoint® H2 User's Guide for proper use of the meter.

A. Instructions For Use (Capillary)

1. Make sure that the meter is ready for use.
2. Make sure that your patient is sitting comfortably.
3. There should be a good blood circulation in the hand from which you wish to take blood, i.e., 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 Alere HemoPoint® H2 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 Alere HemoPoint® H2 Microcuvette completely. Avoid "milking" the finger.
9. Hold the center of the Alere HemoPoint® H2 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 Alere HemoPoint® H2 Microcuvette and take another sample using a new Alere HemoPoint® H2 Microcuvette.



10. In order to avoid contamination of the Alere HemoPoint® H2 Microcuvette holder, remove surplus blood from the outside of the Alere HemoPoint® H2 Microcuvette.
11. The Alere HemoPoint® H2 Microcuvette sample prepared in this way can now be measured immediately or within 10 minutes at the latest.

B. Instructions For Use (Venous or Arterial)

1. Make sure that the meter is ready for use. See the Alere HemoPoint® H2 User Manual for additional information.
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 Alere HemoPoint® H2 Microcuvette from the container and close the lid immediately.
5. Pipette a sufficient drop of blood on a non-absorbent material (e.g. plastic film).
6. Hold the center of the Alere HemoPoint® H2 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 Alere HemoPoint® H2 Microcuvette and take another sample using a new Alere HemoPoint® H2 Microcuvette.
7. In order to avoid contamination of the Alere HemoPoint® H2 Microcuvette holder, remove surplus blood from the outside of the Alere HemoPoint® H2 Microcuvette.
8. The Alere HemoPoint® H2 Microcuvette sample prepared in this way can now be measured immediately or within 10 minutes at the latest.

9. Limitations of the Procedure

1. The Alere HemoPoint® H2 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 Alere HemoPoint® H2 Microcuvette cavity, may cause false results. Discard the Alere HemoPoint® H2 Microcuvette and take another sample using a new Alere HemoPoint® H2 Microcuvette.
3. Ensure that you do not hold the Alere HemoPoint® H2 Microcuvette at its filling end because this may contaminate the optical eye.
4. In order to avoid contamination of the Alere HemoPoint® H2 Microcuvette holder, remove surplus blood from the outside of the Alere HemoPoint® H2 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.
7. Carboxyhemoglobin and turbidity due to leukocytosis or hyperlipemia do not interfere.
8. Always place Alere HemoPoint® H2 Microcuvette right side up in holder. Placing the Alere HemoPoint® H2 Microcuvette upside down can lead to erroneous results.

10. Expected Values^{4,5,6,7,8}

The following hemoglobin values are considered normal:

Adult males:	13.0 – 18.0 g/dL
Adult females:	11.0 – 16.0 g/dL
Children:	11.0 – 16.0 g/dL
Infants (postnatal):	10.0 – 14.0 g/dL

Due to a wide range of conditions which affect normal values, it is recommended that each laboratory establish its own “normal” range.

11. Quality Control

The Alere HemoPoint® H2 AutoCheck performs an internal check of the meter’s optic system every time the Alere HemoPoint® H2 Microcuvette holder is opened. If additional regulatory quality control



is required in your facility, the following checks may be performed: (1) If a Alere HemoPoint® H2 Control Cuvette was supplied with the Alere HemoPoint® H2 Meter it can be used for a simple check of the Alere HemoPoint® H2 Meter's calibration. (2) Use of external controls to assure that the Alere HemoPoint® H2 Microcuvettes and the Alere HemoPoint® H2 Meter are performing correctly. For this purpose, we recommend the use of the Alere™ Hemoglobin Controls, Ref. No. 88772.

Do not use cyanmethemoglobin standards with this test.

12. Results

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

13. Performance Characteristics

A. Precision

Within-run precision using the Alere HemoPoint® H2 Meter with the Alere HemoPoint® H2 Microcuvettes is 2%. The precision evaluation was carried out in accordance with NCCLS EP5-A.⁹ On each of 20 testing days, two separate runs with duplicate measurements within each run were carried out. Three commercially available control materials were used. The test was carried out using: (6) Alere HemoPoint® H2 devices; (16) lots of Alere HemoPoint® H2 Microcuvettes; and (3) operators.

B. Correlation

Correlation coefficient of the Alere HemoPoint® H2 System compared to the NCCLS H15-A3 reference method.

Venous blood: $r = 0.998$

NCCLS EP5-A Protocol	Alere HemoPoint® H2 Microcuvette measured in Alere HemoPoint® H2 device
Hgb/Low (8.0 g/dL): Within-run Precision Total Precision	S_{wr} 0.058 g/dL, CV 0.7% S_T 0.122 g/dL, CV 1.5%
Hgb/Normal (11.8 g/dL): Within-run Precision Total Precision	S_{wr} 0.070 g/dL, CV 0.6% S_T 0.162 g/dL, CV 1.4%
Hgb/High (15.7 g/dL): Within-run Precision Total Precision	S_{wr} 0.087 g/dL, CV 0.6% S_T 0.174 g/dL, CV 1.2%
Day-to-Day Precision	8.0 g/dL: SD 0.111 g/dL, CV 1.4% 11.8 g/dL: SD 0.176 g/dL, CV 1.5% 15.7 g/dL: SD 0.179 g/dL, CV 1.1%

Alere HemoPoint® H2 System (Alere HemoPoint® H2 microcuvettes measured in Alere HemoPoint® H2 device):

Regression line and correlation coefficients compared to NCCLS H15A3 reference method (g/dL), venous blood	1. $Y = 0.2929 + 1.0086x$
	2. $R = 0.998$
	3. $N = 100$, duplicate measurements
	4. Summary of results



14. References

1. NCCLS, Reference and selected procedures for the quantitative determination of hemoglobin in blood – second edition; approved standard, NCCLS Document H15-A3, 2000.
2. G. Vanzetti, An azide-methemoglobin method for hemoglobin determination in blood, *Am. J. Lab. & Clin. Med.* 67 (1966) 116.
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5. DeMott Wayne R., Tilzer Lowell L, Hematology. In: Jacobs DS, DeMott WR, Finley PR, Horvat RT, Kasten Jr BL., Tilzer LL, eds. *Laboratory Test Handbook*. Hudson: Lexi-comp, 1992: 517-626.
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8. Tietz N, ed. *Clinical Guide To Laboratory Tests*, WB Saunders, 1983:258-259.
9. NCCLS, Evaluation of Precision Performance of Clinical Chemistry Devices; Document EP5-A, Vol 19 No. 2, February 1999.

