

# HemoPoint® H2 Microcuvettes

## Procedure No. 3010

For the quantitative determination of hemoglobin in capillary, venous or arterial whole blood.

**CLIA Complexity: Waived**

### Intended Use

The HemoPoint® H2 microcuvettes are intended to be used in the HemoPoint® H2 Photometer and the HemoCue® B-Hemoglobin Photometer. The reagents/microcuvettes and the photometer form an analytical system.

### Summary and Principle

The HemoPoint® H2 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. HemoPoint® H2 cuvettes can also be used in combination with the HemoCue® photometer.

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 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  $\text{NaN}_3$  and thus he was able to reduce the toxicity of the reagent mixture considerably<sup>2</sup>. Vanzetti's method is also known as the azide methemoglobin method. A modified azide methemoglobin method is used in the HemoPoint® H2 system.

### Principles of the Procedure

In the HemoPoint® H2 system, the use of microcuvettes with short light pathways makes it possible to analyze undiluted blood. The filled microcuvette is inserted into the HemoPoint® H2 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 10  $\mu\text{L}$  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  $\text{NaNO}_2$  to trivalent iron, in methemoglobin. Existing and formed methemoglobin and azide ions from sodium azide  $\text{NaN}_3$  form a colored complex which exhibits maximal absorption at 540 and 575 nm and hence it can be quantitatively determined photometrically.

### Reagents

#### HemoPoint® H2 Microcuvettes, Cat. No. 3011

40% w/w sodium deoxycholate, 20% sodium azide, 20% w/w sodium nitrite and 20% w/w non-reactive ingredients.

### Warnings and Precautions

Microcuvettes are designed for *in-vitro* diagnostic use only. The reagents which coat the inner walls of the 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 potential infectious waste per your local regulations.

### Storage

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 cuvettes 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 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.

### Sample Collection and Preparation

The HemoPoint® H2 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°C).

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

Refer to the HemoPoint® H2 User's Guide (or manual of the HemoCue® instrument) for proper use of the photometer.

### Materials Provided

HemoPoint® H2 Microcuvettes, Cat.No. 3010-050

### Materials Required But Not Provided

HemoPoint® H2 or HemoCue® Photometer<sup>3</sup>

HemoPoint® H2 or HemoCue® Control Cuvette

HemoPoint® H2 Hemoglobin Controls, (Cat. No. 3060-601)

Disposable pipettes (venous or arterial blood only)

Plastic film (venous or arterial blood only)

Lint-free material

### Instructions For Use (Capillary)

1. Make sure that the Photometer is ready for use. See the HemoPoint® H2 User's Guide or HemoCue® Operating Manual for the device.
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, 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** User's Guide or HemoCue® Operating Manual 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 cuvette sample prepared in this way can now be measured immediately or within 10 minutes at the latest.

### Limitations of the Procedure

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.

### Expected Values<sup>4,5,6,7,8</sup>

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.

## Quality Control

The **HemoPoint® H2** AutoCheck performs an internal check of the photometer's optic system every time the cuvette holder is opened. If additional regulatory quality control checks are required, the following checks are recommended. (1) The control cuvette supplied with the photometer can be used for a simple check of the photometer's calibration. (2) Good Laboratory Practices recommend the daily use of external controls to assure that the microcuvettes and the photometer are performing correctly. For this purpose, we recommend the use of Stanbio's **HemoPoint® H2** Hemoglobin Controls, Cat. No. 3060-601. Do not use cyanmethemoglobin standards with this test.

### Use of HemoPoint® H2 Cuvettes on HemoCue® Analyzer

To ensure correct performance, validation of HemoCue® Analyzer with the **HemoPoint® H2** cuvettes is required initially and each time the instrument is serviced or its software is upgraded. This validation is accomplished using the Stanbio **HemoPoint® H2** Hemoglobin Controls. The controls are tested on the instrument and the obtained values must be within the established ranges. If values are outside the ranges, contact Stanbio's Technical Service.

## Results

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

## Performance Characteristics

### Precision

Within-run precision using the **HemoPoint® H2** and the HemoCue® devices with the **HemoPoint® H2** microcuvettes is 2%. The precision evaluation was carried out in accordance with NCCLS EP5-A<sup>9</sup>. 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) **HemoPoint® H2** devices; (2) HemoCue® devices; (16) lots of **HemoPoint® H2** microcuvettes and (3) operators.

### Correlation

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

Venous blood:  $r = 0.98$

Correlation coefficient of the **HemoPoint® H2** microcuvettes on the HemoCue® device compared to HemoCue® System.

Venous blood:  $r = 0.97$

NCCLS 5 EP5-A Protocol	H2 cuvette measured in <b>HemoPoint®</b> device	H2 cuvette measured in HemoCue® device
Hgb/Low (10.7 g/dL): Within-run Precision Total Precision	$S_w$ 0.095 g/dL, CV 0.9% $S_b$ 0.114 g/dL, CV 1.1%	$S_w$ 0.068 g/dL, CV 0.6% $S_b$ 0.086 g/dL, CV 0.8%
Hgb/Normal (12.9 g/dL): Within-run Precision Total Precision	$S_w$ 0.084 g/dL, CV 0.7% $S_b$ 0.148 g/dL, CV 1.1%	$S_w$ 0.102 g/dL, CV 0.8% $S_b$ 0.134 g/dL, CV 1.0%
Hgb/High (17.3 g/dL): Within-run Precision Total Precision	$S_w$ 0.111 g/dL, CV 0.6% $S_b$ 0.207 g/dL, CV 1.2%	$S_w$ 0.103 g/dL, CV 0.6% $S_b$ 0.162 g/dL, CV 0.9%
Day-to-Day Precision	10.7 g/dL: SD 0.102 g/dL, CV 1.0% 12.9 g/dL: SD 0.141 g/dL, CV 1.1% 17.3 g/dL: SD 0.169 g/dL, CV 1.0%	10.9 g/dL: SD 0.094 g/dL, CV 0.9% 13.0 g/dL: SD 0.126 g/dL, CV 0.9% 17.2 g/dL: SD 0.148 g/dL, CV 0.9%

**HemoPoint® H2** System: (**HemoPoint® H2** cuvettes measured in **HemoPoint® H2** device):

Regression line and correlation coefficients compared to NCCLS H15A3 reference method (g/dL), venous blood	1. $Y = 0.023 + 1.006x$ 2. $R = 0.999$ 3. $N = 174$ , duplicate measurements 4. Range 3.31 g/dL to 24.4 g/dL 5. Summary of results from (4) Clinical Sites
Regression line and correlation coefficients compared to HemoCue® system (g/dL), venous blood	1. $Y = 0.223 + 1.001x$ 2. $R = 0.998$ 3. $N = 286$ , duplicate measurements 4. Range 3.25 g/dL to 23.85 g/dL 5. Summary of results from (4) Clinical Sites

**HemoPoint® H2** microcuvettes measured in HemoCue® device<sup>10</sup>:

Regression line and correlation coefficients compared to HemoCue® system (g/dL), venous blood	1. $Y = 0.139 + 0.986x$ 2. $R = 0.999$ 3. $N = 286$ , duplicate measurements 4. Range 3.25 g/dL to 23.85 g/dL 5. Summary of results from (4) Clinical Sites
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## 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.
3. **HemoPoint® H2** Hemoglobin testing system User's Guide, Stanbio Laboratory, Boerne, Texas USA.
4. Fandek N, Moreau D, Newell KC, Ofner A, eds. *Clinical Laboratory Tests – Values and Implications*. 2<sup>nd</sup> ed. Springhouse Corporation, 1995:328p.
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.
6. Wallach J. eds. *Interpretation of Diagnostic Tests – A Synopsis of Laboratory Medicine*, 4<sup>th</sup> ed. Boston/Toronto: Little Brown and Co. 1986:6p.
7. Painter Pennell C, Cope June Y, Smith Jane L, Appendix. In: Burtis CA, Ashwood ER, eds. *Tietz Textbook of Clinical Chemistry*. Philadelphia: WB Saunders, 1994:2161-2217.
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.
10. HemoCue® Blood Hemoglobin Photometer, Operating Manual, HemoCue AB Ångelholm, Sweden.  
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