

HemoCue Hb 201⁺ METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose of this document is to describe the procedure for performing a Haemoglobin (Hb) test using the HemoCue Hb 201⁺ analyser. The HemoCue Hb 201⁺ meter can be used by healthcare professionals for measuring Hb. This method is to be used for the determination of haemoglobin in venous, arterial or capillary whole blood using the HemoCue Hb 201⁺ meter.

2. HAZARDS

Patient Samples

All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Personal Protective Equipment (e.g. gloves and safety glasses) should be worn when processing samples, quality control testing and maintenance procedures.

3. CLINICAL SIGNIFICANCE

Anaemia is defined as a low concentration of haemoglobin. Haemoglobin is the main component of red blood cells (RBCs) that transports oxygen from the lungs to other tissues and then returns carbon dioxide from the body to the lungs for removal.

Anaemia occurs when RBCs are lost through excessive bleeding, if the bone marrow fails to produce enough RBCs or when there is premature destruction of RBCs in the circulation. Symptoms of anaemia depend on the aetiology but commonly include fatigue, muscle weakness, shortness of breath, rapid heartbeat and pale skin.

Anaemia can be caused by vitamin or mineral deficiencies particularly of iron, vitamin B12 and folic acid. Anaemia can also be a result of underlying diseases such as cancer, rheumatoid arthritis, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and chronic kidney disease. In approximately 25% of cases no cause can be identified.

Treatment for anaemia is based on the underlying cause and usually involves dietary changes, nutritional supplements and medications such as epoetin for patients with chronic kidney disease or cancer-related anaemia. In severe cases of anaemia, blood transfusions may be necessary.

4. TEST PRINCIPLE

Within the microcuvette, sodium deoxycholate haemolyses the red blood cells releasing haemoglobin. Sodium nitrite then converts the haemoglobin to methemoglobin which, together with sodium azide, gives azide methemoglobin. The absorbance of the sample is measured at two wavelengths (570 and 880 nm) in order to compensate for turbidity in the sample. The haemoglobin level is then calculated by the meter and displayed on the screen.

4.1 Interference

- Mixing samples for extended periods can produce increased oxygen pressure and viscosity that may give false results.
- Values above 235 g/L must be confirmed using a suitable laboratory method.
- Following substances have not been found to interfere: Acetaminophen (1322.8 $\mu\text{mol/L}$), ascorbic acid (170.3 $\mu\text{mol/L}$), conjugated bilirubin (648.2 $\mu\text{mol/L}$), unconjugated bilirubin (648.2 $\mu\text{mol/L}$), creatinine (2.65 mmol/L), ibuprofen (1.94 mmol/L), leukocytes ($600 \times 10^9/\text{L}$), lipaemia (intralipid 4000 mg/L , triglycerides 11.3 mmol/L), salicylic acid (36.2 $\mu\text{mol/L}$), tetracycline (20 mg/dL), thrombocytes ($2100 \times 10^9/\text{L}$), urea (178.5 mmol/L), uric acid (1.19 mmol/L) and pH between 6.3 – 9.0 have not been found to interfere with the system.

4.2 Accuracy

The results of the comparison studies between the HemoCue Hb 201⁺ system and the International Council for Standardization in Haematology method (ICSH)³ are summarized in the table below. The study was performed on four HemoCue Hb 201⁺ Analyzer which had not been recalibrated during the study period.

Study	N	Min g/dL	Max g/dL	Regression line	Correlation coefficient (r)
1	498	4.1	20.5	$Y=1.009X - 0.008$	0.998
2	103	9.0	17.7	$Y=0.971X + 0.584$	0.934

1=ICSH (Cyanmethemoglobin method), venous EDTA blood, multicenter study
 2=ICSH (Cyanmethemoglobin method), capillary blood

4.3 Precision

Within-run precision was determined according to the NCCLS Document EP5-A⁵. The results given below in “Within-run precision” and “Total precision” come from 1 batch of HemoCue Hb 201 Microcuvettes and 5 HemoCue Hb 201+ Analyzers. No recalibration was performed during the analyzing period. Commercially available controls at 2 different levels were used. The hemoglobin concentration was measured in duplicate twice a day, morning and afternoon, during 20 consecutive days.

Control Level	N	\bar{x} g/dL	Within-run Precision SD g/dL	Within-run Precision CV %	Total Precision SD g/dL	Total Precision CV %
1	400	7.70	0.057	0.74	0.100	1.30
2	400	15.36	0.078	0.51	0.109	0.71

Additionally, a study has been performed to determine the precision using fresh blood samples. The study was performed on venous whole blood samples tested in duplicate.

5. INSTRUMENT



Product specifications

5.1 Operating and technical conditions

Temperature range	15°-30°C
Relative humidity	<90% (non-condensing)
Position	Place meter on a level, vibration-free surface
Measurement range	0-256 g/L
Test time	15-60 seconds
Memory	600 test results with date and time
Barcode scanner	No
Interface	The instrument is made for continuous mode
Battery operation	Four AA batteries or power adapter recommended by HemoCue or CE marked adapter
Mains connection	Power supply adapter: Input: 100-240 V / 50-60Hz / < 500 mA
Automatic power-off	To turn off the analyser press and hold the left button until the display reads OFF and becomes blank

Dimensions	85 x 160 x 43 mm
Weight	350g

5.2 Storage and transport conditions

Temperature range	15°-30°C
Relative humidity	<90% (non-condensing)

6. SPECIMEN REQUIREMENTS

6.1 Sample Material

Venous or arterial blood is recommended for use on the HemoCue Hb instrument. Bloods must be collected into an EDTA or heparin tube. Capillary samples can be used but results should be interpreted with caution.

Venepuncture (see suitable anticoagulants above)

- If the blood has been stored in a refrigerator it must be allowed to reach room temperature before analysis.
- Haemoglobin remains unchanged for days, provided that the blood does not become infected.

Capillary blood (from finger prick)

- **Wash hand with warm soapy water and dry thoroughly prior to collecting samples**
- Capillary samples can be used but should be interpreted with caution as inaccurate results may be produced due to poor circulation.
- If the result does not fit the clinical picture, repeat using a venous sample.

7. CARTRIDGES/REAGENTS

7.1 Storage and handling

- The microcuvettes are stored at room temperature. Do not refrigerate.
- Individually packaged microcuvettes are stable until their expiry date.
- The microcuvette is for single use only.

8. CALIBRATION

The HemoCue Hb 201⁺ instrument is calibrated against the international reference method for haemoglobin determination, ICSH³.

9. QUALITY CONTROL

Quality control material (perform as per your organisation protocol)

Accurately testing known levels of haemoglobin ensures that the system and your technique used in testing give accurate results on patient tests. The

control solutions have defined (known) values. The results for these solutions must first fall within a certain acceptable range in order to allow valid patient testing.

A quality control test should be performed every time a new shipment of test cuvettes are received, when a new lot number of test cuvettes are used, if the clinical picture does not correlate with the patient test results, after major maintenance, and at a minimum of once a month.

Enrolling in an External Quality Assurance Program is encouraged to objectively compare results with other users using the same method of testing. If an External Quality Assurance Program is not available, monthly lab comparison is encouraged.

9.1 Internal quality control

The HemoCue Hb 201⁺ uses the HemoTrol quality control solutions (three levels), each with a known haemoglobin concentration. Quality control material should be stored refrigerated at all times when not in use (2-8°C). The control solution is stable for 30 days after opening or until the expiry date (whichever occurs first).



9.2 Performing the Test

- Remove vials from the refrigerator for 15 minutes to come to room temperature
- Roll the vial back and forth for 20 - 30 seconds. Occasionally invert the vial to mix the control solution. Mix vigorously, but do not shake.
- Continue to mix in this manner until the red cells are completely suspended. Vials stored for a long time may require extra mixing.
- Gently invert the vial 8 - 10 times immediately before sampling
- Remove the cap from the vial. Dispense a drop of control solution on to a hydrophobic surface e.g. plastic film or inside of the microcuvette package
- Fill the microcuvette in one continuous process. Place the microcuvette in the analyser.
- The result will be displayed on the instrument when the test is complete.
- Ensure this falls within the expected range indicated on the insert sent with your control vial. If the result does not fall within the expected range, repeat the test.

9.3 Laboratory Comparison

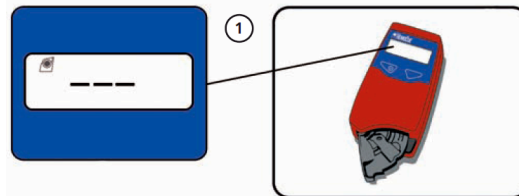
If your policy states you must perform laboratory comparison, then perform a venepuncture and test the sample on the HemoCue Hb 201⁺ and send it to the laboratory for comparison. Record and compare the results ensuring they are in acceptable range for your organisation

10. TEST PROCEDURE

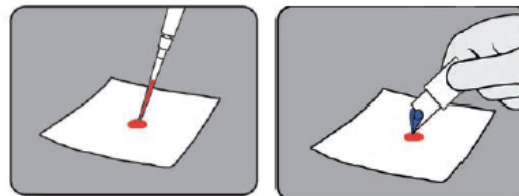
10.1 Performing the Test

Collecting the sample

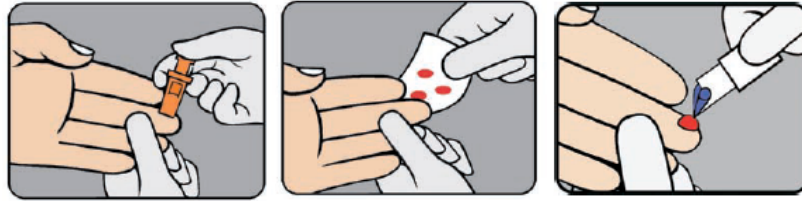
- Turn on the instrument. After start-up the cuvette holder should be in its loading position. The display will show three flashing dashes and the HemoCue symbol.



- If using a venous or arterial sample, mix the tube well by inverting 8-10 times before testing. Place a drop of blood on to a hydrophobic surface e.g. plastic film, or inside of the microcuvette package and fill the microcuvette in one continuous process. Follow the test procedure from 'Applying blood to the cuvette'.

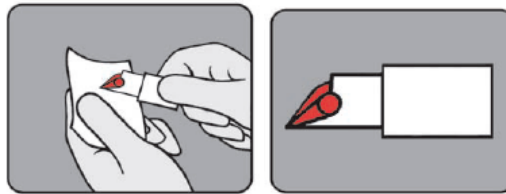


- If using capillary samples, results should be interpreted with caution. Make sure the patient's hand is warm and relaxed. Use only the middle or ring finger for sampling and avoid fingers with rings on.
- Clean finger with disinfectant and allow to dry.
- Using your thumb, lightly press the finger from the top of the knuckle towards the tip. This stimulates the blood flow towards the sampling point.
- For best blood flow and least pain sample at the side of the fingertip, not in the centre.
- Whilst pressing lightly towards the fingertip, prick the finger using a lancet
- Wipe away the first two or three drops of blood.
- Re-apply light pressure towards the fingertip until another drop of blood appears.
- When the drop is large enough, fill the microcuvette in one continuous process, filling from the tip of the microcuvette. **DO NOT REFILL.**



Inserting the cuvette

- Wipe off excess blood on the outside of the microcuvette tip using a tissue. Make sure no blood is drawn out of the microcuvette during this procedure.



- Look for air bubbles in the filled microcuvette. If bubbles are present take a new sample. Small bubbles around the edge can be ignored.

Inserting cuvette into the meter

- Place the filled microcuvette in the cuvette holder. Testing should be performed within 10 minutes from filling the microcuvette.
- Push the cuvette holder to its measuring position.
- During the measurement, the hourglass symbol appears on the screen.
- After 15-60 seconds the haemoglobin value of the sample is displayed. The result will remain on the display as long as the cuvette holder is in the measuring position. When operating on battery power the analyser will automatically turn off after approximately 5 minutes.
- Once the test is completed, discard the used microcuvette in the hazard bin.

11. RESULTS

Measuring range

The instrument has a measuring range of 0 – 256 g/L. Results above 256 g/L are displayed as 'HHH'.

Accessing previous results

- The analyser automatically stores 600 results. When the memory is full the analyser will automatically overwrite the oldest result.
- The most recent result in the memory is displayed first
- Press the left or right button to scroll backwards or forwards between the results. The stored values are shown in the display. When the button is held down the user can see the order of the results, the most recent

measurement is always number 1 and when the button is released the result is shown.

Reference Range

Adult males 130 – 170 g/L

Adult females 120 – 150 g/L

Infants, after neonatal period 110 – 140 g/L

Children, two years to teenage: gradual increase to adult normal.

Additional information for healthcare professionals

If the haemoglobin results do not reflect the patient's clinical symptoms, or seem unusually high or low, perform a control test. If the control test confirms that the system is working properly, repeat the test. If the repeated result still seems unusual, follow facility guidelines for further action. Your policy may encourage sending the specimen to the laboratory for testing.

12. MAINTENANCE

16.1 Cleaning the cuvette holder

- At the end of each day's use, remove the cuvette holder and clean with alcohol or a mild soap solution.
- The cuvette holder can also be autoclaved.
- It is important that the holder is completely dry before being replaced in the meter.

16.2 Cleaning the optronic unit

- Clean the optronic unit using the HemoCue Cleaner when directed to do so by an error message.



- Check that the analyser is turned off. The display should be blank.
- Pull the cuvette holder out to its loading position. Use a pointed object to carefully depress the small catch positioned in the upper right corner of the cuvette holder.
- Whilst keeping the catch depressed, carefully pull the cuvette holder in the direction in which the handle of the cuvette holder is pointing.
- Clean the cuvette holder with alcohol or mild detergent.

- Push the HemoCue Cleaner swab into the opening of the cuvette holder. Pull out and push in 5 –10 times. If the swab is stained, repeat with a new swab. Stop when the swab comes out clean.
- Wait 15 minutes before re-using the analyser. Replace the cuvette holder. The cover may be cleaned with alcohol or a mild soap solution.

13. REFERENCE SOURCE OF METHOD

Adapted from the method described in the HemoCue Hb 201⁺ manual and HemoCue Hb 201⁺ Microcuvette package insert.