

Hemoglobin Determination by HemoCue Analyzer

I. Purpose:

"Anemia is said to exist when the level of circulating hemoglobin in the patient is lower than that of healthy persons of the same age group and sex in the same environment"(WHO). The most common type of anemia is iron deficiency resulting from inadequate iron intake. The objective of this procedure is to measure the amount of Hemoglobin in peripheral whole blood.

II. Specimen:

The preferred site for collection of capillary blood sample is from the middle or ring finger of children or adults. The WIC program does not recommend obtaining a specimen from the ear lobe. Infants (less than one year old) who have not begun to walk may have blood collected from the heel. Blood obtained by finger stick (lancet) must be free flowing and not forced; do not "milk" the finger to get sufficient blood. Wipe away the first and second drop with a clean, dry gauze or lint-free tissue and then collect the third drop of blood for analysis. Do not use cotton balls since cotton fibers will interfere with the test.

III. Safety:

- A. Since blood is a primary carrier for hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV), standard (universal) precautions are required. Wear appropriate personal protective equipment including gloves and lab coat.
- B. Dispose of finger stick devices and microcuvettes in a sharps container.
- C. Dispose of all blood soaked items as biohazardous waste.

IV. Materials:

- A. Instrument
 - 1. HemoCue® Hemoglobin Photometer
 - 2. HemoCue® transformer (5 AA alkaline batteries)
 - 3. HemoCue® manual
- B. Supplies
 - 1. Microcuvettes (Hemocue®, catalog # 651200) (200/box)
 - 2. Hemocue® Red Control Cuvette
 - 3. Gloves
 - 4. Alcohol, lint free tissues (e.g., Kimwipes), gauze squares, or alcohol pledgets
 - 5. Blood lancet & Biohazard/sharps container
 - 6. Disinfectant
- C. Controls: Hemocue has determined that the following controls are compatible with their instrumentation. Each site must choose which of these control materials to order and use at their laboratory.
 - 1. BioRad Meter Trax TM : Low (catalog # 75961) and High (catalog 75963)

2. R&D Glu/Hgb Controls: R&D Systems, Inc., Minneapolis MN Low (catalog #GH00L) and High (catalog #GH00H)
3. HEMA-Trol™ Whole Blood Hgb Controls: Nerl Diagnostics, East Providence, RI. Low (catalog # HYC84666) and High (catalog # HYC84668).
4. HemoTrol: Eurotrol, 800-323-1674 (available for purchase through Hemocue). Low (catalog # 022.001.002) and High (catalog # 022.003.002).

D. Storage conditions:

1. Microcuvettes: Store at room temperature (15-30 °C or 59-86 °F) in a dry place. Unopened microcuvettes have an expiration date specified by the manufacturer and printed on the outside of the container. Once opened, the microcuvettes are stable for three (3) months. Tightly reseal container immediately after use. Write both the date opened and the open vial expiration date on the container.
2. Instrument: Remove the batteries from the HemoCue analyzer if it will be stored for more than a week.
3. Controls: Refer to the manufacturer package insert for specific storage criteria.
 - a. Unopened controls are stable until the expiration date specified by the manufacturer when stored in the refrigerator (2-8°C or 35-46°F).
 - b. Once opened, the controls vials may be stored in either the refrigerator or at room temperature, provided they are handled properly.
 - c. Once opened, write both the date opened and the open vial expiration date on the vial.
 - d. The length of time a control is stable after opening (the open vial expiration date) is specified by the manufacturer. Read the package insert and strictly adhere to the manufacturer's guidelines. For example:
 - 1) BioRad Meter Trax Controls are stable for 31 days after opening when stored in either the refrigerator or at room temperature
 - 2) R&D Glu/Hgb are stable for 30 days after opening when stored in either the refrigerator or at room temperature.
 - 3) HEMA-Trol Controls are stable for 60 days after opening if stored in the refrigerator or for 30 days if stored at room temperature.
 - 4) HemoTrol (Eurotrol) controls are stable for 30 days after opening when stored at room temperature. This material has a 12 month shelf life after manufacture.

- e. If controls are stored in the refrigerator, they must be returned promptly to the refrigerator after testing.
- f. If controls are stored at room temperature, they must be kept in a closed container (e.g., zip-lock bag) since they are a blood product.

V. Quality Control

- A. Both a high and a low control must be used each week of testing before any patient samples are tested. This will ensure that the instrument is functioning properly, that the microcuvettes are capable of accurately detecting critically low (anemia) and unusually high hemoglobin levels, and that the analyst is performing the test accurately.
- B. A daily optic check must be performed using the red control cuvette when using the Hemocue Hemoglobin B analyzer.
NOTE: The Hemocue 201 analyzer does not require a daily optic check since it has been calibrated at the factory. Refer to regional lab procedure RL.37.01 if your facility uses the Hemocue 201 analyzer.
- C. Frequency:
 - 1. A high control and a low control must be run once a week on each Hemocue instrument in the laboratory prior to patient testing.
 - 2. A high and low control must be run to check each new vial of microcuvettes before they are used for client testing.
 - 3. A high and low control must be run to check each new lot number of microcuvettes before they are used for client testing.
- D. Responsibility for running weekly controls must be distributed among all staff so that each analyst will be performing QC on a regular basis. The use of a “designated QC analyst” who performs all QC activities at a clinic is not permitted.
- E. Carefully mix the control reagents while bringing them to room temperature. The blood cells should not cling to the bottom of the vial when they have been properly mixed. NEVER SHAKE vials. An easy way to get a good control sample is to place one drop of control solution on a hydrophobic material such as parafilm or a clean glass slide and then touch the drop of the control blood with the tip of the microcuvette.
- F. Expected values:
 - 1. The maximum acceptable range for each control will be listed on a specification sheet included with each set of vials. The analyst must ensure that both the daily optic check and weekly controls are within the expected range before analysis of patient samples is performed.
 - 2. Retain a copy of the manufacturer’s package insert for each lot number of control solutions so that it is possible to verify that the

expected range of the control matches the lot number and expected ranges entered on the laboratory QC sheet.

G. Corrective action

1. The optics check and controls must be repeated if the observed results fall outside accepted limits.
2. If the optics check consistently fails to read according to specifications, notify your supervisor and call HemoCue technical service for further instructions. DO NOT test any patient samples if the optic check fails.
3. Contact information
 1. Tech Support: 800-352-3252 9:00am - 5:00pm EST
 2. HemoCue T.S.: 800-426-7256 9:00am - 5:00pm PST
4. If the low and high controls fail to read within expected range, they should be repeated. A second failure suggests that either the cuvettes are damaged or the liquid controls have been damaged. A troubleshooting outline can be found in the Quality Control: An Overview@ guideline. *Hint: when the photometer assembly is dirty, the controls will often be out of range. Clean the interior chamber and repeat the analysis.*
5. If the Quality Control checks fail, it is important to note that corrective action was taken on the QC Log sheet.

H. Maintenance schedule

1. Clean the exterior of the instrument on a daily basis with a clean cloth which has been slightly dampened with mild detergent. Quaternary ammonium disinfectants or Lysol™ may be used, but should be removed with a cloth dampened with water.
2. Clean the interior of the photometer and lens assembly daily (or each day that the instrument is in operation) with a long stem cotton-tipped applicator that has been saturated with water. Squeeze out the excess water and clean the inside of the analyzer until no traces of blood are seen. Alcohol is not to be used in cleaning the interior of the photometer and lens assembly.
3. The microcuvette holder should be cleaned daily with either soap and water or with alcohol.
4. The Red Control Cuvette may be washed with soap and water if it appears that it has picked up any blood from the instrument.
5. The Red Control Cuvette and microcuvette holder should be cleaned at the end of each day that testing is performed so that they will be ready for use the next morning.
6. For more thorough cleaning instructions, refer to the HemoCue Cleaning and Maintenance procedure (RL.21.01).
7. When not in use, keep the Red Control cuvette in its case. Store at room temperature in a dry location.

VII. Method

- A. Optic Check- performed each day of testing.
1. Attach the AC-adaptor to the photometer power inlet (use only the adaptor provided with the HemoCue instrument).
 2. Plug the AC-adaptor into the wall outlet.
 3. Turn the HemoCue power switch to the ON position, located on the back of machine.
 4. The letters "Hb" should be seen on the photometer.
 5. Pull the black microcuvette holder out until you hear a "click"; then stop.
 6. After approximately 15 seconds the indication "READY" will appear on the display together with three flashing dashes.
 7. Confirm that the serial number of the photometer matches the serial number of the Red Control Cuvette. Write the serial number of the photometer on the Daily QC log.
 8. Place the Red Control Cuvette into the cuvette holder and push in toward machine; the display will show "Measuring" and three fixed dashes.

NOTE: Do not discard or lose the Red Control Cuvette. The serial number of the Red Control Cuvette must match the serial number on the back of the Hemocue instrument. The Red Control Cuvettes are instrument specific and are not interchangeable and are not replaceable without charge (approximately \$80).

9. After approximately 10 to 15 seconds, the value of the Red Control Cuvette will be displayed.
 10. Compare the value of the Red Control Cuvette with the expected range specified by the manufacturer and listed on the control microcuvette storage case. This value must be within +/- 0.3gm/dL of this value. If the value is outside of the expected range, repeat the test in 30 minutes. If the problem is still unresolved call technical service numbers listed under corrective action.
 11. Record the result on daily Quality Control Log sheet.
 12. Remove the Red Control cuvette and return it to the storage case.
- B Test procedure for weekly QC
1. Warm both control (low and high) vials to room temperature for 15-30 minutes before mixing.
 2. Hold vial upright and roll the vial slowly between the palms several times. Do not pre-mix on a mechanical mixer.
 3. Gently invert the vial 8-10 times immediately before sampling.
 4. Inspect vial contents to ensure that cells have been uniformly distributed.

NOTE: If the control is not uniformly distributed, the dilution of the vials may be altered, affecting the accuracy of the test result.

5. Repeat steps 2-4 if uniform distribution is not observed.
6. Remove cap from vial. Dispense one drop of the control onto a non-permeable surface (e.g., an unopened alcohol wipe, piece of parafilm, glass slide, etc.).
7. Hold the microcuvette at the “wing” end and touch the tip into the middle of the drop of control. Fill in one continuous process. Do not refill a partially filled microcuvette.
8. Wipe any residual control material from the sides of the microcuvette with a piece of gauze, as if wiping excess butter from a knife. Do not touch the opened end with the gauze since this will draw blood out.

NOTE: The control solutions are different from human blood. Therefore it is essential to wait 1-2 minutes after loading the microcuvette and placing the microcuvette into the holder before pushing the sample into the instrument and taking a reading.

9. Visually inspect for air bubbles in the center of the cuvette eye. If bubbles are present in the eye, discard the microcuvette and obtain another specimen.
10. Place the filled microcuvette into the holder and gently slide the holder into the measuring position in the instrument. Filled microcuvettes are to be kept in the horizontal position.
11. The hemoglobin value will be displayed in grams/dL after approximately 30-50 seconds.
12. Document the result on the QC sheet.
13. Remove the microcuvette from the instrument and discard into a biohazard sharps container.
14. Verify that all controls (daily optic check and weekly low and high external controls) are within acceptable limits before testing any patient samples.

C. Test procedure for patient testing

1. Seat the patient comfortably. For best results, use the middle finger or the ring finger for sampling. Avoid fingers with rings for sampling.
2. If cold, warm patients fingers with warm water. The patient's fingers should be straight but not tense, to avoid stasis.
3. Remove a microcuvette from the vial and recap immediately.
4. Clean site for blood collection with alcohol-soaked gauze or a newly-opened alcohol pledget.
5. Using your thumb, lightly press the finger from the top knuckle to the tip to stimulate flow of blood to the sampling point. For the best blood flow and the least pain, sample at the side of the fingertip, not the center.
6. Position the lancet device so that the puncture will be made across the whorls (lines) of the fingerprint. Press the lancet firmly off-

- center on the fingertip prior to activating the lancet to aid in obtaining a good sample.
7. Activate the lancet to puncture the fingertip. Discard the lancet in an approved sharps container.
 8. Wipe away the first two large drops of blood. This stimulates the blood flow and lessens the likelihood of a dilutional effect by interstitial fluid. If necessary, apply light pressure again, until another drop of blood appears. Avoid “milking of the finger”.
 9. Make sure the **third** drop of blood is big enough to fill the microcuvette completely. Hold the microcuvette at the “wing” end and touch the tip into the middle of the drop of blood from above the finger. Keep the microcuvette in contact with the blood and fill in one continuous process. Do not refill a partially filled microcuvette.
 10. Wipe any residual control material from the sides of the microcuvette with a piece of gauze, as if wiping excess butter from a knife. Do not touch the opened end with the gauze since this will draw blood out of the microcuvette.
 11. Visually inspect for air bubbles in the center of the cuvette eye. If bubbles are present in the cuvette eye, discard the microcuvette and obtain another specimen.
 12. The filled microcuvette should be analyzed within three (3) minutes after loading. Filled microcuvettes are to be kept in the horizontal position. Place the filled microcuvette into the cuvette holder and gently slide the holder into the measuring position.
 13. The Hemoglobin (hgb) value will be displayed in grams/dL after approximately 30-50 seconds.
 14. Record the result before removing the microcuvette from the instrument.
 15. Dispose of the microcuvette in the biohazardous waste container.
 16. If an “ERROR” code is displayed, refer to the manufacturer’s “Troubleshooting Guide” found on page 16 of the Hemocue operating manual.
 17. If the patient is a child, place a Band-Aid on the puncture site only if there is no indication that the child may choke on a removed bandage. To avoid choking on the Band-Aid, use procedures such as reminding the caretaker to watch the child closely to be sure the bandaged finger is not put in the child’s mouth. The Band-Aid can be removed before leaving the clinic. The objective is to avoid choking and merely asking if the child sucks on his/her fingers is not sufficient.
 18. Remove gloves and wash hands.
NOTE: A sink with running water must be accessible for immediate hand washing before and after applying and removing gloves for taking blood samples.

- C. Criteria for repeat analysis of patients with critically low hemoglobin values.
1. Section 4.04 of the WIC Policy and Procedure Manual (issue date 2/11/02) provides guidance for testing of women and children.
 2. Section 2.04 of the WIC Policy and Procedure Manual (issue date 10/1/05) lists the cutoff values for low hemoglobin values for women and children.
 3. Children with initial hemoglobin results between 10.0 and 11.0 must be rechecked in six months.
Note: WIC agencies only recertify every six months; the policy for WIC is to retest every six months that the test is below the accepted range until a normal result is found. At that time the child may be checked annually as long as the test remains in the normal range.)
 4. Children with initial hemoglobin results of 10.0 or lower must be rechecked immediately.
 - a. Repuncture at a new site and recheck the hemoglobin immediately.
 - 1) Document both results on the patient test log.
 - 2) Document the second result on the WIC form in the patient chart if it is within normal limits. Most low readings are either due to air bubbles in the microcuvette or poor technique on the part of the analyst.
 - 3) If the repeat result is still below the cut-off limit, document the result of the repeat test on the WIC form in the client chart. Low readings (below the cut off values in policy) will be referred to the participant's primary care provider for a confirmatory venous test and any needed follow-up.
 - 4) A third repeat is not recommended.
 - b. If the reported value is below 11.0 but above 10.0, recheck the hemoglobin at the next certification visit in six months, in accordance with WIC policy.
 - c. If the reported hemoglobin value is less than 10.0, the following guidance is recommended:
 - 1) Refer the client to physician/primary care provider and reporting the lab value.
 - 2) Assess dietary iron intake and provide nutrition education to assist the client in achieving adequate nutrition.
 - 3) Refer for chart review with RD to evaluate growth and diet if the client has a diagnosis of anemia or fails to show improvement in hemoglobin values.

- 4) Repeat the hemoglobin screening test in six months in accordance with WIC policy.
 - d. Children with abnormal readings will be referred to their primary care provider for a confirmatory venous test.
 5. Adults with initial hemoglobin results that are considered to be below the critical laboratory value for hemoglobin (as defined by either WIC policy or the local health department).
 - a. Repuncture at a new site and recheck the hemoglobin immediately. Document both results on the patient test log.
 - b. Document the second result in the patient record if it is closest to the normal range and technician technique on the first test was suspect.
 - c. A third repeat is not recommended.
 6. In most cases, if the first screening test is low but the second test result is within the normal range, the problem may usually be attributed to faulty technique of the tester in collection of the first sample. A common problem is the introduction of air into the microcuvette. Excessive milking of the finger may lead to the introduction of air bubbles or dilution of blood with serous fluid.
- C. Notification of panic values
 1. Each local agency performing hemoglobin testing must have a written policy which establishes the critical range of hemoglobin results at that site. A critical range is defined as the hemoglobin levels which present an immediate potential health threat and must be addressed immediately by a physician or other qualified health provider.
 2. Each site must have a written policy for physician notification of abnormal client conditions. This policy must include the steps which must be taken so that the patient's primary care provider is immediately notified of results that fall below the critical laboratory value for hemoglobin.
 3. The laboratory must maintain documentation verifying that the physician was notified of a critical laboratory value for hemoglobin.

VII. Limitations of method

- A. The quality of the sample has a considerable effect upon the accuracy and precision of this test. Examine the sample drawn into the microcuvette; bubbles of any size in the circular optical eye are unacceptable - throw the microcuvette away and get another sample.
- B. Exposure to moisture in room air will inactivate the reagents in the microcuvette; take only enough microcuvettes out of the canister that you will use within 5 to 10 minutes; do not return any microcuvettes to the storage canister. Keep the canister lid tightly closed.

- C. Values above 23.5 gm/dL must be confirmed using a suitable laboratory method.
- D. Sulfhemoglobin is not measured with this method.

VIII. Procedural Notes

- A. The Hemocue® Hemoglobin photometer corrects for turbidity in specimens and therefore might produce lower results than those expected for other hemoglobin instruments that do not have this correction feature. Therefore, only controls that are assayed for the Hemocue® Hemoglobin system are recommended.
- B. Results above 25.6 gm/dL will be displayed as ERROR 999 or ERROR HHH. Refer to the Trouble Shooting Guide in the Operating Manual for interpretations of other error codes.

VIII. Sources Error and Recommended Actions

- A. Bubbles in the sample: The quality of the sample has a considerable effect upon the accuracy and precision of this test. Examine the sample drawn into the microcuvette; bubbles of any size in the circular optic window are unacceptable. If bubbles are present, discard the microcuvette and take another sample.
- B. Microcuvettes exposed to moisture: Exposure to moisture in room air will inactivate the reagents in the microcuvette, causing a discoloration. Microcuvettes must be used within 5 to 10 minutes of being taken from the canister. After removing microcuvettes from the canister, replace the cap immediately, taking care to assure it is tightly sealed.
- C. Microcuvettes used 90 days after opening canister: The cuvettes are stable for 90 days after opening a new canister. Date the canister when opened and write the new expiration date on the label. Discard unused microcuvettes after 90 days. The individually wrapped microcuvettes available for the Hemocue 201+ have a longer shelf life.
- D. Microcuvettes exposed to excessive temperatures: Store microcuvettes in their canister at room temperature. Monitor the temperature of the room in order to detect fluctuations of temperature which may have a detrimental impact on the integrity of the microcuvettes.

IX. Results

- A. Normal Values²:

Population	Hemoglobin
Infants (full term)	10.5 – 19.5 g/dL
Children (1-9 years)	11.0 – 14.0 g/dL
Children (10-12 years)	11.5 – 15.0 g/dL
Men (Adults)	13.0 – 18.0 g/dL
Women (Adults)	12.0 – 16.0 g/dL
Pregnant women	11.0 – 14.0 g/dL

B. Hemoglobin Ranges for Anemia: Children under 5 and pregnant women

Mild Anemia	9.0 – 11.0 g/dL
Moderate Anemia	7.0 – 9.0 g/dL
Severe Anemia	< 7.0 g/dL
Very Severe Anemia	< 4.0 g/dL

C. Hemoglobin Ranges for Anemia: Children over 5 and nonpregnant women

Mild Anemia	9.0 – 12.0 g/dL
Moderate Anemia	7.0 – 9.0 g/dL
Severe Anemia	< 7.0 g/dL
Very Severe Anemia	< 4.0 g/dL

D. WIC Criteria for critical values – women (WIC Policy and Procedure Manual, Section 2.04, effective 10/1/05)

Status	Non-smoking	Smoking up to 19 cigarette/day	Smoking 20-39 cigarette/day	Smoking 40 or more cigarette/day
Post-partum - at/over age 15	<12.0	<12.3	<12.5	<12.7
Post-partum – under age 15	<11.8	<12.1	<12.3	<12.5
1 st Trimester (0-13 weeks)	<11.0	<11.3	<11.5	<11.7
2 nd Trimester (14-26 weeks)	<10.5	<10.8	<11.0	<11.2
3 rd Trimester (27 wks or more)	<11.0	<11.3	<11.5	<11.7

E. WIC Criteria for critical values – infants and children ((WIC Policy and Procedure Manual, Section 2.04, effective 10/1/05)

Status	
Infant: 6 – 11 months	<11.0
Child: 12 - 23 months	<11.0
Child: 24 – 59 months	<11.1

X. Records

A. Clinic Logs:

1. Sufficient information must be recorded to insure that the client is adequately identified and that one can trace the result from the clinic log to the client record.
2. Record the lot number and expiration date of the microcuvettes on the daily patient log.
3. Record the patient result on a daily patient log.

B. QC Records:

1. Record the serial number of the Hemocue photometer.
2. Record the expiration date and lot number of the microcuvettes, low and high controls, and the Red Control Cuvette on the QC log sheet.
3. Enter the observed values and expected values for the optic check, low control and high control.
4. The analyst must document the acceptability (pass or fail) of the daily optic check and weekly high/low controls.
5. The site coordinator will sign the QC log at the end of the month. The QC log will be sent to the Laboratory Director or Technical Consultant for review and signature. Retain a copy of the QC log until the original is signed and returned.
6. QC logs must be filed for two years before they may be discarded.

XI. References

1. HemoCue Instruction Manual
2. Wallach, J. Interpretation of Diagnostic Tests, 2nd ed., pg-6, Table 2., Little, Brown and Company, Boston MA 02106
3. The WIC User Guide. Appendix C, page C1-4. Issued 6/89. Michigan Department of Public Health. Lansing, Michigan.
4. Anemia Detection Methods in Low-Resource Settings: A Manual for Health Workers. 1997. US Agency for International Development
http://www.path.org/files/TS_anemia_guide_health_workers.pdf

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