HemoCue® Plasma/Low Hb Procedure

PURPOSE

The HemoCue Plasma/Low Hb system is used for the quantitative determination of low levels of hemoglobin in plasma and serum specimens, aqueous solutions, stored or banked erythrocytes using a specially designed photometer, the HemoCue Plasma/Low Hb photometer and specially designed microcuvettes, the HemoCue Plasma/Low Hb microcuvettes.

PRINCIPLE

Although there are numerous methods for measuring low levels of hemoglobin in plasma, serum, aqueous solutions, etc., there is no widely accepted method, and results can vary considerably between these methods.

The HemoCue Plasma/Low Hb system is factory calibrated to the International Reference Method for hemoglobin testing, ICSH.

The hemoglobin concentration is determined as azidemethemoglobin utilizing a microcuvette with a dry reagent system and a dual wavelength photometer. When present, the membranes of erythrocytes are disintegrated by sodium deoxycholate, releasing hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with sodium azide to form azidemethemoglobin. Measurements are taken at 570nm and at 880nm; the latter to correct for turbidity.

SAMPLE COLLECTION AND PREPARATION

Plasma, serum and aqueous solutions containing low concentrations of hemoglobin, such as irrigating fluid from surgical procedures, stored or banked erythrocytes may be used.

If assaying for “free” hemoglobin, caution should be used when separating the supernatant from the erythrocytes to avoid in-vitro hemolysis or contamination with intact red cells.

EQUIPMENT, REAGENTS, AND SUPPLIES

- HemoCue® Plasma/Low Hb Photometer
- HemoCue® Plasma/Low Hb Microcuvettes (store at room temperature, keep vial tightly closed when not in use). Open bottle stability is 3 months. Write expiration date on bottles.
- Eurotrol Plasma/Low Hb Controls levels 1 and 2 (store refrigerated at 2-8 deg C when not in use)
- Lint free tissue
- Hydrophobic material such as Parafilm®
- Pipettes and disposable pipette tips or disposable transfer pipettes
- Filter with pore size of 0.2µm
- Gloves
- cotton swabs
Note: We presently do not have a back up HemoCue photometer. If analyzer is down or out of supplies, inform the floor that the sample will be sent out and it will take 2-3 days to get the result. Please use the following procedure:

1. Reprint a sticker label and aliquot the sample using the false-bottom tube.
2. Give the aliquot sample to Central Processing so they can order “MOLT” for Hemoglobin, Free, Plasma to Quest Diagnostics. Test Code is #514. Stable for 7 days refrigerated or 30 days frozen.
3. Notify the unit and send the ETC “PFHU” (Rapid plasma hemoglobin testing unavailable due to analyzer malfunction or reagent shortage. Sample will be sent out to a reference lab.) in Sunquest then append the name of the person taking the information, their phone number, your initials, the time and date of the call and credit the test.
4. Make sure to inform the supervisor or specialist so he/she can notify the laboratory director if needed.

**PROCEDURE**

A. **Start Up Procedure**

1. Turn the photometer on using the switch in the back.
2. Pull out the cuvette holder to the loading position. This will be noted by a distinct stop.
3. After about 15 seconds the display screen will show “READY” with three flashing dashes.
4. The photometer is now ready to perform a measurement.

B. **Quality Control**

<table>
<thead>
<tr>
<th>CONTROL NAME</th>
<th>SAMPLE TYPE</th>
<th>STORAGE</th>
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<tbody>
<tr>
<td>Eurotrol levels 1 and 2 vials in use kept refrigerated when not in use. Once open, vials are stable for 30 days at 2-8 deg C. Write expiration date on vials. Control preparations and acceptance of QC results are in “Policies and Procedures” manual</td>
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The two levels of Eurotrol Plasma/Low Hb control are to be included with each run of patient samples.

1. Open a vial of Plasma/Low Hb microcuvettes, removing only the number of cuvettes for immediate use. Recap the vial.
2. Allow control vials to sit at Room Temperature for 15 minutes. Gently mix control vials 8-10 times before sampling. Dispense a drop of control onto a hydrophobic surface.
3. Introduce the tip of the cuvette into the middle of the drop of control blood and allow to fill completely, in one continuous motion. Do not refill a partially filled cuvette.
4. Wipe off the outside of the cuvette with a clean, lint free tissue, taking care not to touch the open end of the cuvette.
5. Visually inspect the cuvette for air bubbles in the optical eye. If bubbles are present in the optical eye, discard the cuvette.
6. Place the filled cuvette in the cuvette holder and gently slide it into the photometer within one minute of filling. The display screen will show “MEASURING” and fixed dashes.
7. The result will be displayed on the screen within one minute.
8. Record the QC results into UCSF Clinical Labs LIS using control codes: PLHB1 and PLHB2 via MEM using worksheet PHGB.

9. Pull the cuvette holder out to the loading position, remove the cuvette and discard it in an appropriate biohazard container.

10. If the control results do not fall within the established acceptable 2SD range, follow UCSF Chemistry section policy for failed quality control action prior to performing any patient testing.

C. Patient and Specimen Testing

Visibly turbid samples should be filtered using a Pall filter with a pore size of 0.2µm.

1. Follow steps 1-9 above using patient specimen, supernatant from erythrocyte suspensions or other suitable sample.

2. Record the results and discard the cuvette as in B#9 above.

**IMPORTANT NOTE:** Patient results MUST BE SENT via MEM using Worksheet PHGB. Test code is PHGBS. Uncorrected patient plasma/low hgb results will be adjusted by calculations programmed within the PHGB worksheet. Record the corrected results next to raw results on the paper worksheet. At UCSF Parnassus Chemistry section ALL patient results are to be corrected by this formula:

\[
\text{Reportable Plasma Hgb result} = (0.907 \times \text{Hemocue readout}) - 20.74
\]

D. Maintenance

1. **Cuvette Holder**
   - The cuvette holder should be removed at the end of each day of use for cleaning.
   - Alcohol or a mild soap solution may be used. The cuvette holder may also be autoclaved. The cuvette holder must be completely dry before reinserting into the photometer.

2. **Photometer Exterior**
   - The exterior may be cleaned with alcohol or a mild soap solution.

3. **Optronic Unit**
   - The optronic unit may be cleaned with the HemoCue Cleaner if available. (Follow instructions in the package).
   - If a HemoCue Cleaner is not available, the optronic unit may be cleaned using a cotton-tipped swab slightly moistened with water. Turn the power off and remove the cuvette holder. Insert the cotton-tipped swab into the photometer about 1½ to 2 inches and gently clean both the upper and lower “cover glasses”. Repeat until the swab no longer picks up any residue. Using a dry swab, dry off the upper and lower surfaces in the optronic unit.

E. Procedural Notes

1. Microcuvettes should be stored at room temperature, away from any direct heat source. The vial should be kept tightly capped. Cuvettes should be removed only as needed for testing just prior to use. Unopened vials of cuvettes are stable until the expiration date printed on the box as well as on each vial. **Vials of cuvettes that have been opened are stable for three (3) months if the vial is tightly capped during storage. Label the vial with the date opened and expiration date.**
2. Controls that are specifically assayed for the HemoCue Plasma/Low Hb System (such as Eurotrol Plasma/Low Hb) are recommended. Do NOT use cyanmethemoglobin controls. Some control blood contains additives that cause the control to be turbid. The HemoCue Plasma/Low Hb photometer corrects for turbidity, and therefore might produce results that are lower than those expected for other instruments that do not have this correction feature.

3. Refer to the HemoCue Plasma/Low Hb Microcuvette package insert and the HemoCue Plasma/Low Hb Operating Manual for additional information and troubleshooting guide.

F. Normal/Reference Values

UCSF Clinical Labs: <8 mg/dL

G. Reportable Range

UCSF Clinical Labs: 8 to 500 mg/dL
Results outside of reportable range: send <8 mg/dL or >500 mg/dL

H. Limitations of the Procedure

1. Air bubbles in the optical eye of the microcuvette may cause false results. If air bubbles are present, discard the cuvette and proceed with a new cuvette.
2. Do not hold the cuvette by the “filling end”. This could result in contamination of the optical eye.
3. Care should be taken to prevent contamination of the optical eye with sample material.
4. For values >3000 mg/dL, use another suitable laboratory method, i.e., the HemoCue β-Hemoglobin System.
5. Normal levels of bilirubin do not influence the assay.
6. Sulfmethemoglobin is not measured with this method.
7. Samples that are visibly turbid should be filtered using a filter with a pore size of 0.2µm.
8. The performance characteristics of this system have not been determined using samples obtained from uremic patients.

I. References

HemoCue Plasma/Low Hb Photometer Operating Manual
HemoCue Plasma/Low Hb Microcuvette Package Insert

For additional information please contact:
HemoCue, Inc.
Attention: Technical Service
40 Empire Drive
Lake Forest, CA 92630
800-426-7256