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| **Plasma Hemoglobin on the Hemocue Low** |
| **Purpose** | This procedure provides instructions for performing Plasma Hemoglobin On The Hemocue Low Hb Photometer. |
| **Policy Statements** | This procedure applies to Chemistry personnel performing Plasma Hemoglobin analysis using the HemoCue Plasma/Low Hb photometer. |
| **Principle** | The HemoCue Plasma/Low Hb system is used for the quantitative determination of low levels of hemoglobin in plasma specimens, aqueous solutions, or 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. 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. |
| **Instrument** | HemoCue® Plasma/Low Hb Photometer |
| **Sunquest Test Code** | **PHG**  |
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| **Materials** | **Reagents, Supplies & Equipment** |
|  | * HemoCue® Plasma/Low Hb Photometer
* HemoCue® Plasma/Low Hb Microcuvettes; store 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 in 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 date of expiration.*
* Eurotrol® Plasma/Low Hb Controls (store and handle according to manufacturer's specifications)
* Lint free tissue
* Hydrophobic material such as Parafilm®
* Pipettes and disposable pipette tips or disposable transfer pipettes
* HemoCue Cleaner or cotton swab
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|  | **Documents** |
|  | * Sunquest Worksheet for Plasma Hgb: PHGB
* [Hemocue Low Maintenance Log](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Forms/204090.pdf)
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| **Sample** | * 0.2 mL heparinized plasma or aqueous solutions containing low concentrations of hemoglobin, such as irrigating fluid from surgical procedures, stored or banked erythrocytes.
* Samples must be collected from a free flowing venipuncture to reduce the potential for in-vitro hemolysis.
* Centrifuge the sample and carefully separate the plasma from the red cells to avoid hemolysis or contamination with intact red cells.
* ***Ultracentrifuge all specimens prior to analysis*** to prevent interference from lipids. After processing, label the specimen as **ultracentrifuged** or **airfuged** to signify the sample has been prepared for testing.
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| **Special Safety Precautions** | * Refer to laboratory safety policies and procedures.
* Dispose of used cuvettes in a biohazardous waste container.
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| **Calibration** | The HemoCue Plasma/Low Hb system is factory calibrated to the International Reference Method for hemoglobin testing, ICSH.Calibration verification is performed biannually by following the procedure [CH 5.40 HemoCue Low Calibration Verification](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Procedure/201782.pdf). |
| **Quality Control** | Two levels of Eurotrol Plasma/Low Hb Control are run once each day of use.* Low Control/Level 1
* High Control/Level 2

Acceptable Ranges: * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the [Westgard Rules in Chemistry procedure](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.18-westgard-rules-in-chemistry.pdf) for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.

Do not load or release patients until QC is acceptable in Unity Real Time. **Stability:** Unopened, stored at 2 - 8° C / date on vialOpened, properly recapped and stored at 2 - 30° C / 30 daysUnacceptable results may indicate deterioration.**Use:**1. Remove vials from refrigerator and allow to warm to room temperature for 15 minutes before mixing.
2. Gently invert the vials 8 – 10 times before sampling.
3. Analyze the samples according to the procedure that follows below.
4. After sampling, clean residual material from the lid with a lint-free tissue, and replace the cap.

If the results do not fall within the established range, prior to performing any patient testing:* 1. Repeat the test
	2. Repeat the test on a fresh vial of control
	3. Clean the optronics, perform routine maintenance, and repeat the test
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| **Maintenance** | **Daily:**Cuvette Holder1. Remove the cuvette holder from the photometer for cleaning.
2. Wipe using an alcohol pad.
3. Dry the cuvette holder before reinserting into the photometer.
4. Document activity on Maintenance Log

**Periodically or As Needed:****Photometer Exterior**Clean the exterior as needed with an alcohol pad, or tissue moistened with water.Optronic Unit1. Turn the power off and remove the cuvette holder.
2. Insert a cotton-tipped swab moistened with purified water into the photometer about 1½ to 2 inches.
3. Gently clean both the upper and lower “cover glasses”. Repeat until the swab no longer picks up any residue.
4. Using a dry swab, dry off the upper and lower surfaces in the optronic unit.
5. Wait 15 minutes after cleaning before replacing the cuvette holder and using the instrument.
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| **Procedures:** |  |
|  | **Step** | **Action** |
| **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. |
| **Running Samples** | 5 | Open a vial of Plasma/Low Hb microcuvettes, removing only the number of cuvettes for immediate use. Recap the vial. |
|  | 6 | Dispense a drop of control or sample onto a hydrophobic surface (parafilm). |
|  | 7 | Introduce the tip of the cuvette into the middle of the drop of blood and allow to fill completely, in one continuous motion. Do not refill a partially filled cuvette. |
|  | 8 | Wipe off the outside of the cuvette with a clean, lint free tissue, taking care not to touch the open end of the cuvette. |
|  | 9 | Visually inspect the cuvette for air bubbles in the optical eye. If bubbles are present in the optical eye, discard the cuvette. |
|  | 10 | 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. |
|  | 11 | The result will be displayed on the screen within one minute. |
|  | 12 | Record the results on the PHGB worksheet. |
|  | 13 | Pull the cuvette holder out to the loading position, remove the cuvette and discard it in biohazard trash. |
|  | 14 | Turn off instrument. |
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| **Interpretation/****Results/Alert Values** | **Manufacturer’s Instrument Range:** 30 – 3000 mg/dL**Reportable Range:** 30 – 1000 mg/dL |
| **Reference Intervals** | Reference Range: 0-30 mg/dLECMO patients:

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| Normal | <30 mg/dL |
| Mildly elevated | 30 -50 mg/dL |
| Moderately elevated | 51 – 70 mg/dL |
| Critical value | >70 mg/dL |

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| Limitations | * 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.
* Do not hold the cuvette by the “filling end”. This could result in contamination of the optical eye.
* Care should be taken to prevent contamination of the optical eye with sample material.
* Bilirubin up to 20 mg/dL does not influence the assay.
* Sulfmethemoglobin is not measured with this method.
* The performance characteristics of this system have not been determined using samples obtained from uremic patients.
* Controls that are specifically assayed for the HemoCue Plasma/Low Hb System 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.
* Refer to the HemoCue Plasma/Low Hb Microcuvette package insert and the HemoCue Plasma/Low Hb Operating Manual for additional information and troubleshooting guide.
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| **Result Reporting** | Results between 30 and 1000 without error messages are reported.* Results < 30 mg/dL will be displayed as a numerical value. Report result as < 30 mg/dL.
* Report all results greater than 1000 mg/dL as > 1000 mg/dL.
* Results >3000 mg/dL will be displayed as “Error HHH”.
* Grossly hemolyzed samples with concentrations >100 mg/dL should be resulted with the Sunquest English Text Code “UNTD”: Unable to determine if hemolysis is in vivo or in vitro in origin.

**Result Reporting (MEM):**1. Print Sunquest Worksheet PHGB and record patient and QC results.
2. In Sunquest, use function MEM.
3. Enter worksheet PHGB.
4. Method = HCUE
5. Enter patient’s accession # and result.
6. Modify the result for all patients. Enter “#” to append ECMO Reference Range composed text.
7. (A) to Accept composed text.
8. (A) Accept or (M) to modify result.
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| **References** | 1. HemoCue Plasma/Low Hb Photometer Operating Manual
2. HemoCue Plasma/Low Hb Microcuvette Package Insert
3. Eurotrol Plasma/Low Hb Control Product insert, Eurotrol, Inc., 563 Main Street, Bolton, MA 01740
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Joan Murrow.  | March 2001 | Initial Version |
| 2 | L. Lichty | 3/1/2005 | Plasma Hemoglobin on FARA |
| 3 | Linda Lichty | November 16, 2006 | Hemocue Plasma Hemoglobin |
| 4 | Linda Lichty | June 19, 2007 | Revised resulting |
| 5 | Linda Lichty | February 10, 2010 | Revised specimen preparation |
| 6 | D. Helfinstine | April 1, 2011 | New format. Renumbered from CH 6.16. |
| 7 | L. Lichty | August 1, 2011 | New quality control material |
| 8 | Erin Bartos | 6/19/2017 | Added labeling of sample after ultracentrifuging, biennial review |
| 9 | Erin Bartos | 05/01/2019 | Updated QC section for URT |
| 10 | Erin Bartos | 2/24/2020 | Removed dual waste disposal- cuvettes are to be disposed of in biohazard waste. |
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