EXERCISE 7: AUTOMATED HEMOGLOBIN TESTING

Objectives:

1. Define the following: hemoglobin, anemia, polycythemia.
2. State two possible causes of anemia and polycythemia.
4. State six criteria which must be considered when setting up the HemoPoint photometer.
5. List 3 sample types and 3 anticoagulants which may be used for testing.
6. State the storage requirements and time limits for use of venous and arterial samples.
7. Describe the testing which must be performed for monitoring quality assurance in hemoglobin testing.
8. State when a “blank reading” is required.
9. State the frequency of testing the “control cuvette” and the importance of only using the control cuvette designated for each instrument.
10. State the proper handling and storage of cuvettes including: temperature, amount of time they can be used after opening and the information to be placed on the container the first time opened.
11. List 8 limitations of the hemoglobin test procedure.
12. State what must be done if hemoglobin values are equal or greater than 23.5 g/dL.
13. State the normal values for hemoglobin levels for adult females, adult males, children and infants.
14. Perform and record the blank reading and testing the control cuvette for the HemoPoint H2.
15. Perform and record the control solutions results for the test.
16. Evaluate the control solutions using predetermined criteria to determine if the instrument can be used for patient testing.
17. Perform an effective capillary puncture.
18. Perform a quantitative measurement of hemoglobin on whole blood using the HemoPoint H2 instrument.
19. Evaluate the patient results and determine whether they fall within the normal range.
20. State the time limit for testing the sample in the microcuvette.
21. Record the results using proper units.
22. State the cleaning procedures for the housing unit, touch screen, cuvette holder and optical unit.
23. State the length of time before the equipment can be used after cleaning the optical unit.
24. State the time limit for using the optical cleaner after removal from the foil pouch.

Discussion

Hemoglobin (Hgb) is the protein in red blood cells that carries oxygen to the cells and returns CO₂ back to the lungs. Hemoglobin gives red cells their color and contains iron. Anemia is a decrease in hemoglobin level and may be the result of recent hemorrhage, fluid retention, or an underlying disease state. Polycythemia is an elevated hemoglobin level due to hemoconcentration from dehydration or excess production of red cells.

Principal of the Procedure

Microcuvettes with short light pathways containing the necessary reagents are filled by capillary action with arterial, venous or capillary blood. The resulting chemical reaction produces a color which is measured by the photometer when the filled cuvette is placed in the instrument, and the result is calculated and displayed.

The HemoPoint® H2 system is intended for the quantitative determination of hemoglobin in whole blood of adults, infants, and children in a professional point-of-care setting. It is designed for use in a variety of inpatient and outpatient settings, such as clinical labs, emergency departments, intensive care units,
medical practices and blood banks. This test system has been approved for use in laboratories with Waived status as defined in 452 CFR 493.15(c)(a).

Setting up the Photometer

Select a suitable place for setting up the photometer, using the following criteria:
1. Avoid direct sunlight
2. Avoid strong electromagnetic fields
3. Avoid direct influence from ionizing radiation
4. Avoid rapid temperature fluctuations (keep away from heaters, open windows, ventilators, fans or air conditioning, etc.)
5. Avoid wet areas (i.e. wash basins)
6. Allow the photometer to reach room temperature! Changing from a cold to a warm environment, (i.e. after storage or transport) condensation can form both on the inside and the outside of the photometer. Wait at least 1 hour before you connect the photometer to a power supply.

Place the photometer on a level counter adjacent to a power socket. Make sure there is enough room for the cuvette holder to be freely accessible. The instrument may be used while plugged into a power source or may be used with the built-in battery. Once the battery is fully charged, it can operate the instrument for approximately 100 hours. If it is not used for some time, it will switch into an energy saving, stand-by mode.

Sample Collection and Preparation

The HemoPoint® H2 Photometer can be used with capillary, venous, or arterial blood. Use EDTA, heparin or heparin/flouride 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 at 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).

Quality Control

The HemoPoint® H2 AutoCheck performs an internal check of the photometer's optic system every time the cuvette holder is opened. Daily use of external controls assures that the microcuvettes and the photometer are performing correctly. The control cuvette supplied with older models of the photometer is used for a check of the photometer's calibration.
Blank reading

An internal check of the photometers optic system is performed every time the cuvette holder is opened. Once the internal check is complete, the instrument will display “Add Cuvette”.

A blank reading is performed by opening the cuvette holder, waiting for the “Add Cuvette” prompt, and closing the holder without inserting a cuvette. The photometer does not start testing but rather runs an electronic check of the instrument. Once the electronic check is complete, the instrument will display “Open Holder”, and the instrument is ready for use.

Always carry out a blank reading whenever you have removed the cuvette holder (i.e., cleaning the cuvette holder, cleaning the optical unit, or replacing with a new cuvette holder). This serves to reset the instruments electronics.

Control Cuvette

Some models of the HemoPoint H2 were supplied with a control cuvette which allows for a simple check of the photometer’s calibration. The control cuvette must be tested daily on these instruments to ensure the testing quality of the photometer. If your instructor provides you with a control cuvette for your instrument, you must run and record those results along with other external controls provided by your instructor. If your instrument does not have a control cuvette, then you can mark those portions of the lab report as NA (Not Applicable).

The Hemoglobin value and the permitted deviation of the control cuvette are stated on the control cuvette storage box label. All photometers have a specific calibration due to the tolerances in the mechanical and electronic components. The control cuvette is only calibrated for the instrument with which it was delivered, i.e. the hemoglobin value stated on the storage box label is only valid for that one photometer, and could lead to completely different results on another photometer.

*If you have several HemoPoint® H2 photometers, keep track of each control cuvette for each photometer. When not being used, keep the control cuvette in the original storage box. It is optimally protected there against breakage and contamination.*

External Quality Control

Controls, when assayed as actual specimens, help a laboratory evaluate whether a given procedure is performing with acceptable accuracy and precision. Laboratory Practices recommend the daily use of external controls to assure that the cuvettes and the photometer are performing correctly.

A set of controls will be provided by your instructor consisting of different levels of control preparations. These are prepared from unfixed, stabilized human erythrocytes containing low and high levels of hemoglobin. These different concentrations can help you assess the precision of the system.

There are a variety of hemoglobin controls on the market today. See package insert for suitability of use with the HemoPoint H2 meter.

Thoroughly mix the controls before use (see the control package insert for directions). *Compare the values obtained with the controls against the stated reference values. If the hemoglobin result falls outside the range of the controls, there is evidently a problem. Notify your instructor, so that the “Troubleshooting” portion of the User Guide can be consulted.*
**Storage and Handling of Cuvettes**
The microcuvettes consist of a clear body with a cavity which takes up approximately 10uL of blood which combines with the dry reagent chemistry. The optical distance between the cuvette walls is fixed and permits photometric determination of hemoglobin in undiluted blood samples.

1. Store the cuvettes in the original container at room temperature 15-30 °C (59-86 °F). The cuvettes are sensitive to moisture and must be kept in the original container with the supplied drying agent. DO NOT refrigerate the cuvettes.
2. Cuvettes are stable for 3 months after opening the container when stored at room temperature.
3. Write the date the vial was opened and your initials on the container label in the space provided. Best practices suggest that the expiration date based on when the container was opened should also be written on the container.
4. Only remove ONE microcuvette at a time from the container and then immediately close the lid.
5. It is crucial not to touch the optical eye of the cuvette with fingers, dirty or sharp objects.
6. The microcuvettes may only be used once and must be disposed after use as potentially infectious waste, in accordance with the current regulations applicable to your establishment. Discard into a biohazard sharps container.
7. The reagents that coat the inner walls of the cuvette are harmful and must not be swallowed.

**Limitations of the Procedure**

1. The microcuvette sample can be measured immediately or within 10 minutes at the latest. After 10 minutes, 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, this may contaminate the optical eye preventing an accurate reading.
4. To avoid contamination of the cuvette holder, remove surplus blood from the outside of the microcuvette.
5. Always place the cuvette right side up in the holder. Placing the cuvette upside down can lead to erroneous results.
6. All results above 23.5 g/dL or equivalent must be confirmed by laboratory methods.

**Expected Values - Normal range/Reference Values**

Adult Males: 13.0 – 18.0 g/dL  
Adult Females: 11.0 – 16.0 g/dL  
Children (2 yrs to teenage): 11.0 – 16.0 g/dL  
Infants (post-natal): 10 – 14 g/dL  

The highest hemoglobin concentrations are usually measured in neonates. Due to the wide range of conditions (dietary, geographical, smoking, exercise, recumbency, etc.), which affect reference values, it is recommended that each laboratory establish its own expected ranges.
PROCEDURES

Performing the Blank Reading

A blank reading is performed at the start of every shift, as it turns the instrument on and runs basic electronic checks. You must also perform a blank reading whenever you have removed the cuvette holder (i.e., cleaning, replacing with a new cuvette holder.)

1. Open the cuvette holder completely.
2. The text in the display reads “Add Cuvette”.
3. Close the cuvette holder, without inserting a cuvette.
4. The photometer runs internal electronic checks. After approximately 2 – 3 seconds the display will read “Open Holder”. If the instrument DOES NOT show “Open Holder”, there is possibly a malfunction. Please consult your instructor and the “Troubleshooting” portion of the User’s Guide.
5. On your lab report, record that you performed the Blank reading.

Testing the Control Cuvette – only performed on those models supplied with a Control Cuvette

1. Open the cuvette holder completely and wait until the photometer displays “Add Cuvette”.
2. Take the control cuvette out of the storage box and place it in the cuvette holder with the curved flange up.
3. Close the cuvette holder completely. The photometer tests the control cuvette and shows the result after a few seconds.
4. Record the target value and expected range from the label of the control cuvette box on your lab report.
5. Compare the result with that stated on the storage box label. The result must lie within the stated range. Record the control result on your lab report!
6. Open the cuvette holder and return the control cuvette to its box.
7. If the Hgb result falls outside the range of the control cuvette, there is evidently a problem. Please notify your instructor and the “Troubleshooting” section of the User’s Guide.
8. If your instrument was not supplied with a control cuvette, mark the Control Cuvette section of your lab report with NA (Not Applicable).

If the original control cuvette is lost or damaged, the photometer must be checked for proper calibration and a new control cuvette must be assayed. The photometer will need to be sent back to Stanbio Laboratory for a new control cuvette assignment. The calibration of the photometer and the re-assay of a new control cuvette is a critical and extensive process. The cost for this is the responsibility of the owner, so please take care of the control cuvette.

Performing External Quality Control

Commercial liquid controls that are recommended by Stanbio must be used on each day of testing to assure proper functioning of the entire system. Follow the manufacturer's procedure for storage and handling of the control material.

1. Allow the vials to come to room temperature 59-86°F for 20 minutes.
2. Mix thoroughly but gently by inverting the vials and repeatedly rolling them between the palms until all cellular components are completely suspended. Do not shake the vial. Check the bottom of the vial to ensure that all cells are completely suspended and not settled in the bottom of the vial. A mechanical mixer is not recommended for the procedure but can be used to maintain the cell suspension.

3. The analyzer should be in the ready mode with the display showing “Open Holder” prior to filling the cuvette.

4. Remove a cuvette from the container, taking care not to touch the optic eye of any of the cuvettes, and immediately close the lid. Carefully lay the cuvette on a clean gauze or bio wipe.

5. Remove the cap from one of the control vials. Dispense a drop of control onto a clean NON-ABSORBENT surface such as plastic film (or the orange side of a bio wipe).

6. Holding the back of the cuvette, bring the center of the cuvette up to the drop of control and allow the cuvette to fill by capillary action. Do not fill from the side of the cuvette as this may result in air bubbles in the optic window and give erroneous results.

7. Using a clean gauze or bio wipe, remove any excess control from the outside of the cuvette by wiping the bottom of the cuvette with a gauze or bio wipe. Do not blot the center of the cuvette where it was filled to avoid removing sample from the cuvette.

8. Open the holder and place the cuvette with the curved flange up on the tray. Close the holder.

9. Control values should be read within 2 minutes after filling the control sample into the cuvette.

10. While waiting for the results, clean the threads of the vial and the vial cap with a cotton or polyester gauze. Snugly recap the vial immediately. After testing, return the vial to its proper storage.

11. Record the lot number, expiration date and expected range of each control on the lab report. Expected ranges for each level are on the package insert which comes with a specific lot number of controls.

12. Record the results you obtained for the control in the quality control section of the lab report.

13. Open the holder and discard the cuvette into a bio-hazard container.

14. Repeat steps 4 – 13 for each level of control.

15. If the results do not fall within the established range, repeat the control test. If results still are outside of established ranges, notify your instructor. Consult the User’s Manual or contact HemoPoint technical support at 800-531-5535. TESTING CANNOT BE PERFORMED IF THE RESULTS OF THE CONTROLS ARE OUT OF RANGE.

Performing Patient Testing

1. Make sure the Photometer is ready for use.

2. Perform the blank reading, test control cuvette (if required), and perform and record quality control. Evaluate the results of quality control to determine if the instrument can be used for patient testing. Record your evaluation on the lab report.

3. Identify yourself and your patient.

4. Disinfect your hands and put on gloves.

5. Explain the procedure to your patient.

6. Make sure that your patient is sitting comfortably.

7. Remove ONE cuvette from the container and IMMEDIATELY close the lid. NOTE: If this is the first cuvette removed WRITE THE DATE and YOUR INITIALS on the container in the space provided. Take care NOT to touch the optical eye of the cuvette with your fingers.

8. There should be good blood circulation in the hand from which you wish to take blood, e.g., it should be warm and relaxed.

9. Gently massage the fingers to stimulate circulation.
10. Disinfect the puncture site and allow to dry.
11. Press lightly on the fingertip with a sampling device and puncture the appropriate area of the finger.
12. WIPE AWAY the first drop of blood then, if necessary, press gently once again to get second drop of blood which is large enough to fill the cuvette completely. AVOID “milking” the finger or using excess pressure.
13. Hold the tip of the cuvette tip in the middle of the drop of blood and let the cavity fill from one drop by capillary action.
14. Visually inspect the cuvette for air bubbles in the optical eye. In case of air bubbles in the optical eye of the cuvette, discard that cuvette and take another sample using a new cuvette.
15. If the cuvette is properly filled, apply a gauze or bio-wipe to the puncture area. Ask the patient to apply pressure to the puncture if possible.
16. To avoid contamination of the cuvette holder, remove surplus blood from the outside of the cuvette by gently wiping with a Biowipe. Do not blot the filling end of the cuvette.
   NOTE: The patient cuvette sample prepared this way can now be measured immediately or within 10 minutes at the latest.
17. Immediately place the cuvette into the cuvette holder with the curved flange up and close the holder. The result will be displayed within 10 seconds to 3 minutes and will remain on the display as long as the cuvette holder is in the measuring position.
18. While the instrument is measuring the hemoglobin, check the puncture site. Once bleeding has stopped, apply a bandage is necessary.
19. Record the patient name and identification number on the lab report. Record the results of the hemoglobin on the lab report USING APPROPRIATE UNITS.
20. After testing is complete, open the cuvette holder and properly dispose of the cuvette in biohazardous trash. Dispose of all other trash in the appropriate receptacle.
21. Thank the patient and allow them to leave. Clean the area with an appropriate disinfectant.
22. Remove gloves and wash hands.
23. Compare the patient results to the stated reference ranges in the lab material to evaluate if the results are lower, normal or higher than expected. Record your evaluation on the lab report.
Maintenance - Cleaning and disinfection of the instrument after use.

Cleaning the Housing and Touch screen
1. Disconnect the power adaptor from the electrical connection before proceeding.
2. Cleaning the housing and touch screen is best accomplished with a lint-free cloth, lightly dampened with clean water. For more stubborn soiling, a mild soap solution may be used. For disinfection, standard solutions can be used for surface disinfection provided they do not contain alcohol or other solvents.

Cleaning the Cuvette Holder
1. The cuvette holder can be removed from the instrument for cleaning. Proceed as follows:
   a. Disconnect the power adaptor from the electrical connection before proceeding.
   b. Open the cuvette holder until you feel a resistance and the holder will not extend further.
   c. Press down the silver pin on the left-hand side (bottom) of the cuvette holder with a ball-point pen and draw the cuvette holder forward at the same time.
2. The cuvette holder can now be cleaned with a mild soap solution. For disinfection, standard solvent-free preparations can be used. DO NOT use any cleaning agent for cleaning the cuvette holder that could leave scratches on its surface.
3. Wait until the cuvette holder is completely dry before returning it to the machine. To replace the cuvette holder, gently push it in the correct position into the opening in the housing until it engages.

Cleaning the Optical Unit
1. The optical unit is situated inside the photometer and has no direct contact with the cuvette therefore no routine cleaning is needed.
2. Cleaning the optical unit can become necessary if the measured maximum light intensity of the photometric light source no longer achieves the appropriate level required for testing. The optical unit should be cleaned when the following error message is displayed: Dirty Optics – Use Optics Cleaner
3. For cleaning the optical unit, the use of a special HemoPoint® H2 optics cleaner is recommended.
4. Remove the cuvette holder following the procedure above for Cleaning the Cuvette Holder.
5. Remove the cleaner from the foil pouch and insert it (narrow tip first) carefully into the opening of the cuvette holder until you feel a smooth resistance. THE CLEANER MUST BE USED WITHIN 10 MINUTES OF OPENING.
6. Slowly push the cleaner deeper into the opening until it stops.
7. Wipe the optical system several times by slowly moving the cleaner in-and-out at least 5 times.
8. Remove the cleaner from the photometer.
9. If the used cleaner is dirty, repeat the procedure with a new cleaner.
10. Wait at least 15 minutes after the optical system has been cleaned to reinsert the cuvette holder into the photometer.
11. Dispose of all used cleaners as potentially infectious waste.
EXERCISE 7: AUTOMATED HEMOGLOBIN TESTING STUDY QUESTIONS

Name___________________________   Date _____________________   Points_________/26

1. Define the following terms (1.5 points):
   a. Hemoglobin:
   b. Anemia:
   c. Polycythemia:

2. State two possible causes of anemia and polycythemia . (2 points)
   a. Anemia
      i. 
      ii.
   b. Polycythemia
      i. 
      ii.

3. State the principle of the HemoPoint H2 hemoglobin procedure. (2 points)

4. State three criteria which must be considered when setting up the HemoPoint photometer. (3 points)
   a.
   b.
   c.

5. List 3 sample types which may be used for testing. (1.5 points)
   a.
   b.
   c.
6. State the storage temperature requirements and time limits for use of blood samples. (2 points)
   a. Storage –
   b. Time limit -

7. State when a “blank reading” of a microcuvette is required. (1 point)

8. State the frequency of testing the “control cuvette”. (1 point)

9. Describe the proper handling and storage of cuvettes including: temperature, amount of time they can be used after opening and the information to be placed on the container the first time opened. (3 points)
   a. Temperature
   b. Time of use after opening
   c. Information on container

10. List 4 limitations of the hemoglobin test procedure. (4 points)
    a. 
    b. 
    c. 
    d. 

11. What must be done if hemoglobin values are equal or greater than 23.5 g/dL. (1 point)

12. State the normal values for hemoglobin levels for adult females, adult males, children and infants. (2 points)
    a. Adult female
    b. Adult male
    c. Children
    d. Infants

13. State the following time limits (2 points total):
    a. Testing the patient sample once it is collected in the cuvette? (0.5 point)
    b. Vials of control to come to room temperature? (0.5 point)
    c. Optical cleaner be used after removal from the foil pouch? (0.5 point)
    d. Instrument to be used after cleaning the optical unit? (0.5 point)
EXERCISE 7: AUTOMATED HEMOGLOBIN TESTING

Phlebotomist ______________________ Date ____________________ Points ____________ /30

Quality Control
1. Perform “blank reading” in accordance with procedure (circle one)  Yes  No

2. Test “control cuvette”  Expected Range for your cuvette:  Your reading:

<table>
<thead>
<tr>
<th>Testing of Controls</th>
<th>Expiration Date</th>
<th>Expected Range (g/dL)</th>
<th>Value Obtained (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Low: Lot #</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. High: Lot #</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Based on the results of the control solutions, can this machine be used? (Circle One)  Yes  No

Sample Collection and Testing (Check P for Performed, NP for Not Performed)  P  NP
6. Role playing: Introduce yourself
7. Role playing: Identify the patient. Record patient name & ID number in chart below
8. Role playing: Explain the procedure.
9. Disinfects hands and put on gloves.
10. Selects, prepares and organizes equipment.
11. Selects puncture site, massages and warms site as necessary,
12. Cleanses site, allows site to dry completely
13. Performs an effective capillary puncture.
14. Appropriately squeezes finger to form first drop of blood.
15. Wipes away first drop of blood.
16. Appropriately squeezes finger to form a second drop of blood.
17. Fills microcuvette using second blood drop without bubbles.
18. Applies gauze or Biowipe to puncture site, asks patient to apply pressure
19. If necessary, wipes off excess blood from microcuvette
20. Places microcuvette in loading tray and inserts into the machine.
21. Reads and records results in table below
22. Provides appropriate post-puncture care to the patient.
23. Disposes of all materials and supplies in the appropriate containers.
24. Inspects puncture site, applies bandage if necessary. Thanks patient.
25. Cleans area with appropriate disinfectant.
26. Removes gloves, washes hands.

Recording and Evaluation of Results

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Patient Identification Number</th>
<th>Results in gm/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Compared the patient results to the reference ranges stated in the lab material.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Are the patient results (CIRCLE ONE) Low Normal High</td>
<td></td>
</tr>
</tbody>
</table>

For Instructor Use Only:
All lines worth 1 point except for Line 27.
Line 27 is worth 3 points total; count off .5 if correct units are not used in the results space.
Count off .25 for each space where correct units are not used on lines 2, 3 and 4.