LESSON 3-3 Bleeding Time
Student Performance Guide

LESSON 3-4 Prothrombin Time
Student Performance Guide

LESSON 3-5 Activated Partial Thromboplastin Time
Student Performance Guide

LESSON 3-6 Rapid Tests for Disorders of Hemostasis
Student Performance Guide
**LESSON 3-3 Bleeding Time**

**INSTRUCTIONS**

1. Practice performing a bleeding-time test (Ivy method) following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the bleeding-time procedure satisfactorily for the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

*Note:* Always follow manufacturer’s directions.

**MATERIALS AND EQUIPMENT**

- gloves
- hand disinfectant
- sterile cotton balls or gauze
- 70% alcohol or alcohol swabs
- blood-pressure cuff
- filter paper
- stopwatch
- surface disinfectant (10% chlorine bleach solution)
- biohazard container
- puncture-proof container for sharps
- butterfly bandage
- commercial bleeding-time device such as Simplate® or Surgicutt®

**PROCEDURE**

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

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<tr>
<th>You must:</th>
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</thead>
<tbody>
<tr>
<td>1. Wash hands and put on gloves</td>
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<tr>
<td>2. Assemble equipment and materials</td>
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<td>3. Explain the procedure to the patient</td>
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<tr>
<td>4. Seat the patient and explain that the test can cause a small scar;</td>
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<tr>
<td>have the patient rest the arm palm side up</td>
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<tr>
<td>5. Choose a site containing no visible blood vessels on the volar surface approximately 5 cm below the cubital crease</td>
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<tr>
<td>6. Cleanse the site with 70% alcohol. Wait for the alcohol to dry completely</td>
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<tr>
<td>7. Place the blood-pressure cuff on the arm above the elbow and inflate to 40mm Hg pressure (for adults). Maintain that pressure during the test</td>
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<td>You must:</td>
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<tr>
<td>8. Set a sharps-disposal container within reach</td>
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<tr>
<td>9. Prepare the incision device following manufacturer's instructions; place the device on the surface of the clean site within 30 seconds of inflating the cuff. The incision should be either parallel or perpendicular to the bend of the elbow</td>
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<tr>
<td>10. Push the trigger to release the blade(s); start the stopwatch</td>
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<tr>
<td>11. Discard the device into the sharps container</td>
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<tr>
<td>12. Touch the filter paper to the drop of blood after 30 seconds; do not touch the wound</td>
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<tr>
<td>13. Repeat step 12 every 30 seconds until no blood appears on the filter paper</td>
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<tr>
<td>14. Stop the watch</td>
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<tr>
<td>15. <em>Remove the blood pressure cuff.</em></td>
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<tr>
<td>16. Wash the site gently with a non-alcohol wipe; when dry, apply a butterfly bandage to minimize the chance of scarring. Advise the patient to leave the bandage in place for twenty-four hours</td>
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<tr>
<td>17. Discard filter paper in biohazard container</td>
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<tr>
<td>18. Wipe the work area with surface disinfectant</td>
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<tr>
<td>19. Remove and discard gloves in biohazard container. Wash hands with hand disinfectant</td>
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<tr>
<td>20. Report results as the time on the stopwatch</td>
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</table>

*Evaluator Comments:*

Evaluator ___________________________________________________________ Date _________________________
INSTRUCTIONS

1. Practice performing the prothrombin time test following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the prothrombin time test satisfactorily for the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

Note: Follow manufacturer’s directions for instrument and reagents used

MATERIALS AND EQUIPMENT

- gloves
- centrifuge
- commercial source of thromboplastin-CaCl₂
- fresh citrated human plasma
- normal controls
- abnormal controls
- distilled water for reconstituting controls
- biohazard container
- surface disinfectant (10% chlorine bleach solution)
- laboratory tissue
- hand disinfectant
- test tube rack

For Manual Method

- test tubes (13 x 75 mm)
- waterbath at 37°C
- pipets to transfer 0.1 and 0.2 mL
- stopwatch

For Automated Method

- coagulation instrument, such as fibrometer
- supplies for instrument

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

You must:    | S | U | Comments
-------------|---|---|------------
1. Wash hands and put on gloves                      |
2. Obtain citrated blood sample (if not provided)    |
3. Centrifuge the specimen as specified in reagent package insert  |
4. Remove the plasma and transfer to a clean test tube. Label with patient identification  |
5. Perform a manual prothrombin time:  
a. Check that waterbath temperature is 37°C  |
You must:

<table>
<thead>
<tr>
<th>Step</th>
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<tbody>
<tr>
<td>b. Pipet 0.2 mL of thromboplastin-CaCl₂ reagent into seven labeled tubes (three for the patient, two for each normal and abnormal control)</td>
</tr>
<tr>
<td>c. Place tubes in rack in waterbath</td>
</tr>
<tr>
<td>d. Pipet sufficient patient plasma and control plasmas (0.4–0.5 mL each) to perform the test in triplicate in another set of appropriately labeled tubes</td>
</tr>
<tr>
<td>e. Place tubes in rack in waterbath</td>
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<tr>
<td>f. Allow patient sample, controls, and reagent to warm for the prescribed amount of time</td>
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<tr>
<td>g. Draw up 0.1 mL patient plasma and forcibly expel into tube containing reagent, starting stopwatch simultaneously</td>
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<tr>
<td>h. Allow tube to remain in waterbath about ten seconds before picking it up and rapidly wiping water from outside of tube</td>
</tr>
<tr>
<td>i. Work quickly—pick up the tube, wipe the outside with tissue, start tilting tube slowly back and forth in front of good light source</td>
</tr>
<tr>
<td>j. Stop the watch at the first sign of thickening (clot) in the moving liquid and record the time</td>
</tr>
<tr>
<td>k. Repeat steps g–j using another tube of warmed reagent. Remember that the first time is approximate, and the second and third should agree with each other</td>
</tr>
<tr>
<td>l. Perform steps g–j in duplicate for each control sample</td>
</tr>
<tr>
<td>m. Report results: Report average of patient’s second and third times; record the times for the controls</td>
</tr>
</tbody>
</table>

6. Perform an automated prothrombin time:
(If instrument is not available, proceed to step 7)

<table>
<thead>
<tr>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Turn on instrument. If using the instrument’s pipetter, be certain it is turned “OFF”</td>
</tr>
<tr>
<td>b. Label desired number of sample cups and place in heat block (patient samples should be run in duplicate)</td>
</tr>
<tr>
<td>c. Pipet 0.2 mL of thromboplastin-CaCl₂ into cups, following manufacturer’s instructions</td>
</tr>
<tr>
<td>d. Pipet sufficient patient plasma and controls (0.4–0.5 mL) into separate cups to allow for duplicate testing of patient and controls</td>
</tr>
<tr>
<td>e. Allow all components to warm the prescribed amount of time. Place one sample cup with measured thromboplastin-CaCl₂ into center well of fibrometer</td>
</tr>
<tr>
<td>f. Draw up 0.1 mL of patient plasma</td>
</tr>
<tr>
<td>g. Turn pipetter “ON”</td>
</tr>
<tr>
<td>h. Expel plasma into center cup containing 0.2 mL thromboplastin-CaCl₂. The timer will start automatically when the plunger is depressed, if using instrument’s pipet</td>
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<tr>
<td>You must:</td>
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<tr>
<td>i. Wait for timer to stop, signaling the formation of a clot</td>
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<tr>
<td>j. Record the time in seconds</td>
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<tr>
<td>k. Gently wipe probe wires with laboratory tissue between determinations</td>
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<tr>
<td>l. Repeat steps e–k using patient plasma</td>
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<tr>
<td>m. Average the two times and report the results</td>
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<tr>
<td>n. Repeat steps e–k for each control</td>
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<tr>
<td>o. Record the times for the controls</td>
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<tr>
<td>p. Turn off instrument</td>
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<tr>
<td>7. Return all equipment to proper storage</td>
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<tr>
<td>8. Dispose of all contaminated articles in biohazard container and</td>
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<tr>
<td>contaminated sharps in sharps container</td>
</tr>
<tr>
<td>9. Wipe counter with surface disinfectant</td>
</tr>
<tr>
<td>10. Remove and discard gloves in biohazard container</td>
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<tr>
<td>11. Wash hands with hand disinfectant</td>
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</tbody>
</table>

**Evaluator Comments:**

Evaluator __________________________________________________________ Date _________________________
LESSON 3-5 Activated Partial Thromboplastin Time

Name _________________________________________________________________ Date ______________________

INSTRUCTIONS

1. Practice performing the APTT following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the APTT satisfactorily for the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

*Note*: Follow manufacturers’ instructions for instrument and reagents used.

MATERIALS AND EQUIPMENT

- gloves
- venipuncture materials required for obtaining citrated blood, or citrated blood sample
- clinical centrifuge
- commercial control plasma
- biohazard container
- puncture-proof container for sharps
- hand disinfectant
- surface disinfectant (10% chlorine bleach solution)
- fibrometer and pipetter (or other coagulation analyzer)
- fibrometer tips and cups
- 37°C heat block
- commercial activated cephaloplastin (partial thromboplastin)
- CaCl₂, 0.02 M

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

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<thead>
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<th>You must:</th>
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<tbody>
<tr>
<td>1. Wash hands and put on gloves</td>
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<tr>
<td>2. Assemble materials and equipment and turn on instrument to warm up</td>
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<tr>
<td>3. Obtain citrated blood sample by venipuncture and label with patient’s name (or use commercial plasma controls)</td>
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<tr>
<td>4. Centrifuge the blood sample for five minutes at 1,500 rpm to obtain the plasma</td>
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<td>5. Follow instructions for the instrument being used. Check to see that heat block is at 37°C</td>
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<tr>
<td>6. Reconstitute the activated cephaloplastin according to manufacturer’s instructions</td>
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S = Satisfactory
U = Unsatisfactory

Estridge, B., Reynolds, A., and Walters, N. Basic Medical Laboratory Techniques. © 2000 Delmar, a division of Thomson Learning
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<tr>
<td>7.</td>
<td>Label instrument cups: normal control, abnormal control, patient name, cephaloplastin, and CaCl₂</td>
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<td>8.</td>
<td>Pipet enough cephaloplastin into reagent cup(s) to have 0.1 mL for each test (which should be run in duplicate)</td>
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<td>9.</td>
<td>Pipet enough control plasma into labeled cups to have 0.1 mL for each test</td>
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<tr>
<td>10.</td>
<td>Pipet into the reagent cups enough CaCl₂ to have 0.1 mL for each test</td>
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<td>11.</td>
<td>Allow reagents and plasmas to prewarm for at least three minutes and not more than ten minutes</td>
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<td>12.</td>
<td>Place a clean cup in the reaction well of the instrument and pipet 0.1 mL cephaloplastin into it</td>
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<td>13.</td>
<td>Perform the APTT on a normal control plasma by pipetting 0.1 mL of normal control into the cup containing the 0.1 mL of prewarmed cephaloplastin (in the reaction well)</td>
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<td>14.</td>
<td>Let the mixture warm and activate for three minutes</td>
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<td>15.</td>
<td>Draw up 0.1 mL prewarmed CaCl₂, turn on pipetter, and dispense CaCl₂ into the cup in the reaction well. The probe will lower into the cup and the timer will start automatically and stop when a fibrin clot is detected</td>
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<td>16.</td>
<td>Record the time from the instrument’s timer</td>
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<td>17.</td>
<td>Repeat the test (steps 13–16) on the normal control plasma</td>
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<td>18.</td>
<td>Average the results and report the APTT in seconds</td>
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<td>19.</td>
<td>Perform the APTT in duplicate using the abnormal control plasma, following steps 13–18</td>
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<td>20.</td>
<td>If all control values are within acceptable limits, repeat steps 13–18, using patient plasma</td>
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<tr>
<td>21.</td>
<td>Dispose of all biohazard waste in biohazard container</td>
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<tr>
<td>22.</td>
<td>Dispose of contaminated sharps in sharps container</td>
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<tr>
<td>23.</td>
<td>Turn instrument off and return all equipment to proper storage</td>
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<td>24.</td>
<td>Wipe counter with surface disinfectant</td>
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<tr>
<td>25.</td>
<td>Remove and discard gloves in biohazard container; wash hands with hand disinfectant</td>
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**Evaluator Comments:**

Evaluator ___________________________________________ Date ____________________
**INSTRUCTIONS**

1. Practice performing a rapid test for fibrin-degradation products or ACT following the step-by-step procedure.

2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the procedure for either fibrin-degradation products or the ACT as directed by the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

**Note:** The following is a general procedure for detecting D-dimer fragments using the DADE® Dimertest® and a general procedure for the performance of an ACT using the HEMOCHRON® Jr. system. The manufacturers’ instructions for the specific methods or instruments being used must be followed.

**MATERIALS AND EQUIPMENT**

- gloves
- marking pencil
- blood-collection equipment:
  - syringe venipuncture (ACT test)
  - vacuum tube blood collection equipment (D-dimer test), including citrate collection tube
- capillary puncture materials
- commercial kit for detecting D-dimer fragments
- pipets and tips to deliver 20 and 100 µL
- disposable plastic test tubes and test tube rack (optional, for semi-quantitative D-dimer test)
- mechanical slide rotator (optional)
- HEMOCHRON® Jr. or other handheld instrument for determining ACT, including cuvettes and whole blood controls
- surface disinfectant
- biohazard container
- biohazard sharps container

**PROCEDURE**

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

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<th>You must:</th>
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<tbody>
<tr>
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<tr>
<td>2. Wash hands and put on gloves</td>
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<tr>
<td>3. Perform the manual test for D-dimers (DADE® Dimertest® Latex Assay). If manual test is not available skip to step 4</td>
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<tr>
<td>a. Obtain plasma from a citrated specimen</td>
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<tr>
<td>b. Allow the reagents and specimen to equilibrate to room temperature</td>
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<tr>
<td>c. Mark patient identifications and control in white areas of the slide</td>
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<tr>
<td>d. Hold the latex beads bottle vertically and add one drop of latex beads to each test area</td>
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### You must:

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<th>Step</th>
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<tr>
<td>e.</td>
<td>Dispense 20 µL of undiluted patient plasma or control solution adjacent to the latex beads in each test area</td>
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<tr>
<td>f.</td>
<td>Promptly mix the suspensions together until the test area is covered; <em>use a new stirrer for each test area and discard after use</em></td>
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<tr>
<td>g.</td>
<td>Rotate the slide gently for three minutes, manually or using a rotator</td>
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<tr>
<td>h.</td>
<td>Exactly three minutes after mixing, check for agglutination (clumping); make observations before the suspension begins to dry out to avoid a false positive reaction. (A positive result is white agglutination on a black background; a negative result is a homogeneous white mixture on a black background)</td>
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<tr>
<td>i.</td>
<td>Record the results for the patient and the positive and negative controls</td>
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<tr>
<td>j.</td>
<td>Optional: if test is positive, perform the semi-quantitative test following the manufacturer’s instructions</td>
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<tr>
<td>k.</td>
<td>If controls are within acceptable limits, report the patient results</td>
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</tbody>
</table>

4. Perform activated clotting time (ACT+) using HEMOCHRON® Jr. (The whole blood controls must be prepared 15 minutes before use; follow the manufacturer’s instructions for rehydration and use)
   - a. Insert the cuvette into the slot to start the instrument
   - b. Obtain a fresh whole blood specimen (1-2 mL)
   - c. Wait for message “ADD SAMPLE” to appear and add the sample to the center well of the cuvette using a syringe, with or without the needle attached
   - d. Fill the well to the top; avoid bubble formation. Allow excess sample to spill over into the outer well
   - e. Depress the START button
   - f. Wait for the result to be displayed
   - g. Repeat steps 4a through 4f for both levels of controls or as directed by the instructor. Refer to acceptable range provided with each quality control kit
   - h. If the controls are within the acceptable range, report the patient results

5. Use safety disposal device to discard syringe needle

6. Discard used slides, stirrers, cuvettes, and syringes in biohazard container

7. Discard used sharps in biohazard sharps container

8. Disinfect the work area with surface disinfectant

9. Remove and discard gloves in biohazard container and wash hands with hand disinfectant

10. Return other equipment to storage

_Evaluator Comments:__

Evaluator ___________________________ Date ___________________________