

DEPMEDS LABORATORY PROCEDURES
DEPARTMENT OF CLINICAL SUPPORT SERVICES
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL
FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCL STANDING OPERATING PROCEDURE 01 November 01

PROTHROMBIN TIME BY MLA ELECTRA 750

1. PRINCIPLE:

- a. The MLA Electra 750 utilizes a photometric detection technique to determine the clotting endpoint. In the Prothrombin Time (PT) test, clotting is initiated by adding tissue thromboplastin and calcium to plasma. This test activates in the extrinsic clotting system and a fibrin clot is formed.
- b. The PT is most commonly used to monitor patients on anticoagulant therapy because of its sensitivity to deficiencies of factors VII and X. Normal clotting time is from 10 to 12 seconds. Since patients on oral anticoagulant therapy (coumarin) are usually medicated, their PT results are 2 to 2 1/2 greater than normal.

2. SPECIMEN:

- a. Collect one part 3.2% (0.109 M) sodium citrate to 9 parts whole blood in a plastic syringe or siliconized glass tube.
- b. Centrifuge within one hour after collection for approximately 15 minutes at 3,300 RPM.
- c. Refrigerate at 2-8°C in a plastic or siliconized tube with stopper.
- d. Test plasma within 4 hours of draw. Specimen may be kept at room temperature if tested within 2 hours.
- e. For extended storage, rapidly freeze plasma at -20°C.

3. REAGENTS AND EQUIPMENT:

- a. Thromboplastin C.

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- (1) Store at 2-8°C.
 - (2) Reconstitute with purified water per manufacturer's instructions.
 - (3) After reconstitution store at 2-8°C; use within 24 hours.
 - (4) Record expiration date and time on label.
- b. Controls.
- (1) Store at 2-8°C.
 - (2) Reconstitute Normal and Abnormal with purified water per manufacturer's instructions.
 - (3) After reconstitution store at 2-8°C; use within 24 hours
 - (4) Record expiration date and time on label.
- c. MLA Electra 750: Check temperature and timer per MLA Initial Set-up SOP
- c. 0.2 mL pipet.
- e. 0.1 mL pipet.
- f. Pipet tips.
- g. MLA cuvettes.
- h. Purified water.
4. QUALITY CONTROL:
- a. Check instrument temperature: $37.2^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.
 - (1) Record on Temperature Chart at beginning of shift.
 - (2) If temperature exceeds 37.7°C or falls below 36.7°C , notify supervisor and Medical Maintenance section.
 - b. Normal and abnormal controls.

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- (1) Run at the start of each shift, at the beginning of each specimen run and when new reagent is used.
- (2) Record all results on PT QC chart.
- (3) If any result exceeds ± 2 standard deviations, rerun control.
 - (a) If with-in range, record and continue run.
 - (b) If it exceeds the range a second time, record results and notify supervisor.

5 PROCEDURE:

- a. Turn on MLA 750. Allow a 5-minute warm-up period or until AT TEMP indicator lights, whichever is longer.
- b. Check that LAMP LEVEL switch is in "B" (middle position).
- c. Prewarm thromboplastin for at least one minute or until the reagent has reached 37°C in the REAGENT reservoir with the magnetic stirring bar for agitation. (See Procedural Notes for incubation times.)
- d. Set Mode Switch to PT.
- e. Pipet 0.1 mL of controls and test plasma, in duplicate, into the bottom of test cuvettes and place into heating block.
- f. Set timer for 4 minutes.
- g. After 4 minutes, place first cuvette to be tested in test station.
- h. Aspirate 0.2 mL of warm thromboplastin and into 0.2 ml instrument pipet (red top) and align pipet over test station. Firmly push pipet plunger and hold for one second to start test.
- i. Record test result.
- j. Repeat step H on duplicate specimen.

6. RESULTS:

- a. Agreement of test results.

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- (1) For times less than 20 seconds the two results should agree within 0.5 seconds.
 - (2) For times that are 20.1-30 seconds the two results should agree within 1.0 second
 - (3) For times greater than 30.1 seconds the two results should agree within 2.0 seconds.
 - (4) If times do not agree, run second set of two cuvettes.
- b. Average the two PT results and report to the nearest tenth.
 - c. Ranges.
 - (1) Normal Range: 10-12 seconds (Should be established by each laboratory.)
 - (2) Critical value: Greater than 27 seconds.
 - d. Abnormal PT results on patients who are not on anticoagulant therapy should be repeated.
 - e. If results are critical, notify physician immediately and document notification.
7. PROCEDURAL NOTES:
- a. Patient tube must be accurately filled; over or under filling can cause erroneous results.
 - b. Ensure all reagents are well mixed and have stood for 15 minutes and are mixed again before testing.
 - c. Allow 20 minutes for 10 mL of refrigerated reagent to come to analyzer temperature, 37°C.
 - d. Thromboplastin should not be held at 37°C longer than 60 minutes.
 - e. Use clean pipet tip on instrument pipette for each test to prevent carryover contamination.
 - f. Ensure that reagent is directed into the bottom of the tube and not run down the side of the cuvette. This provides proper mixing of the specimen and reagent.

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g. Patient plasma should not be incubated longer than 5 minutes.

8. LIMITATIONS:

a. Operating temperature range: 10 to 32°C.

b. Storage temperature range: -40 to 55°C.

c. Operating humidity range: 0-60%.

e. Storage humidity range: 0-90%.

9. REFERENCES:

a. Brown, B.A., Hematology: Principles and Procedures. 6th ed., Philadelphia: Lea and Febiger, 1993.

b. MLA Electra 750 Instruction Manual, Medical Laboratory Automation, Inc., 270 Marble Ave. Pleasantville, NY 10570-2982, 1990.