APPENDIX VII - SERUM BACTERIOSTATIC & BACTERICIDAL TITRES

I. Introduction

In the treatment of a patient with bacterial endocarditis or osteomyelitis, it is often important to know whether the prescribed dosages of Antimicrobials are achieving blood levels sufficiently high enough to kill the causative organism.

The bacteriostatic level is the dilution of serum that inhibits visible bacterial growth; the bactericidal level is the serum dilution that kills 99.9% of the initial inoculum.

NOTE: This test is to be performed only with the approval of a microbiologist.

II. Specimen Collection

The dose, the time the dose was given, and the time of collection must be recorded on the requisition. Pre- and post-dose blood specimens are obtained in serum separator tubes. The pre-dose blood specimen is drawn immediately before administering the next dose of Antimicrobial in order to evaluate the pre (trough) level. Blood for the post-dose (peak) level should be drawn 1 hour after an intravenous infusion has been started, 1 hour after an intramuscular dose and 1 to 2 hours after an oral dose.

III. Reagents/Materials/Media

Mueller Hinton Broth (MHB) (100 mL)
Blood Agar (BA)
Sterile 13 x 100 mm glass tubes
Sterile 10 mL pipettes
Sterile yellow pipette tips
Test tube racks
Pipetter
Precision pipette to deliver 20 μL

IV. Procedure

A. Processing of Specimens

Upon arrival in the laboratory, centrifuge the blood and aseptically transfer the serum into a sterile vial.
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B. Preparation of bacterial suspension:
   Inoculate several colonies of a pure culture of the patient's organism (overnight subculture) into 5 mL MHB. Incubate on a shaker at 36°C for a minimum of 3 hours or until it achieves turbidity greater than the 0.5 McFarland standard (approximately 1.3 x 10^8 CFU/mL).

C. Serum dilution:
   a. Place 12 sterile test tubes in a rack for each serum sample to be diluted.
   b. Number the tubes 1 to 12.
   c. Aseptically pipette 1.0 mL of patient's serum into tubes 1 and 2.
   d. Aseptically pipette 1.0 mL of MHB into tubes 2 -12.
   e. With a new 1.0 mL sterile pipette transfer 1.0 mL of serum from tube 2 to tube 3. Mix well.
   f. Serially dilute the serum by sequentially transferring 1.0 mL of the mixture through to tube 10. Discard 1.0 mL of the mixture from tube 10. No serum is to be added to tube 11 (positive inoculum control) or to tube 12 (broth sterility control). The final dilution of serum in tube 10 is 1:512 and final volume in all tubes should be 1.0 mL.

D. Inoculating Broth
   a. Using the Vitek colourimeter, dilute the bacterial suspension to the turbidity of the 0.5 McFarland standard using MHB.
   b. Prepare a 1:4 dilution of the standardized inoculum by adding 1.0 mL of inoculum to 3.0 mL MHB. Mix well.
   c. Using a precision pipette, dispense 20 µL (0.02 mL) of diluted inoculum into tubes 1 through 11. To inoculate, insert the pipette tip well under the surface of the Antimicrobial containing serum broth mixture. AVOID ANY CONTACT BETWEEN THE TIP AND THE WALLS OF THE TUBE - to prevent transfer of organisms to the inside of tube above the meniscus. Mix by flushing 2 or 3 times without creating air bubbles or splashing. Use a new tip for each tube.
   d. Incubate all tubes at 37°C for 20 hours in a CO₂-free incubator.
   e. From the 1:4 dilution of the standardized inoculum, dilute 1:250 in MHB (0.1 mL in 24.9 mL MHB) to achieve an inoculum of 10^5 CFU/mL.
   f. Perform a colony count to confirm the bacterial count in the final inoculum. Transfer 0.001 mL of diluted inoculum to BA by using a urine loop and distribute evenly on the surface of a BA plate.
   g. Incubate the BA plate overnight at 35°C.
Determination of serum bacteriostatic titres

1. After incubation, tube 12 (broth sterility control) should be clear while tube 11 (positive inoculum control) should be turbid.

2. Record the colony count. The colony count plate should have 75-150 colonies. If the colony count is $<75$ or $>150$ consult the charge technologist before reading the tubes.

3. The highest dilution of serum that completely inhibits visible growth represents the bacteriostatic titre.

Determination of serum bactericidal titre

1. Vortex all tubes without visible growth for 15 seconds.

2. Use a urine loop to subculture all of the clear tubes onto 1/4 BA. Incubate at 37°C for 18 hours.

3. After incubation, read the plates and record the colony count.

4. The first dilution showing 99.9% killing activity (ie. no growth on sub-culture) is reported as the serum bactericidal titre.

V. Reporting Results

Telephone all results when available. Report as follows and give a copy of the report to the microbiologist:

- Pre-dose serum bacteriostatic titre -
- Pre-dose serum bactericidal titre -

- Post-dose serum bacteriostatic titre -
- Post-dose serum bactericidal titre -

VI. Reference