BACTEC™ 9000 SEEDED BLOOD CULTURE PREPARATION

I. PRINCIPLE

Currently many inspection/accreditation agencies require that prior to the routine use of a new test, the new test procedure should be validated against a reference method. It is difficult when evaluating a blood culture instrument to inoculate both test method and reference method blood culture vials with the same patient sample. This document outlines a simple procedure for evaluating the ability of blood culture media to support the growth and detection of typical clinical blood pathogens, allowing the validation to be performed with seeded suspensions versus patient specimens.

II. SPECIMEN

Normal, sterile saline containing an organism suspension of 10-100 CFU.

III. MATERIALS

1. ***BACTEC*** 9000 Series Blood Culture Medium
2. ***BACTEC*** 9000 Series Blood Culture Instrument
3. Reference blood culture medium
4. Test tubes of Normal, sterile saline - of sufficient size (to fit turbidity meter)
5. Test tubes of Normal, sterile saline - 10 mL
6. Plated Media Products appropriate for the organism: TSA with 5% sheep blood, Chocolate Agar, Sabouraud Dextrose Agar
7. 70% isopropyl alcohol pads
8. Sterile 1 mL pipettes for tube dilutions
9. Sterile 1 mL syringes for vial inoculation
10. Subculturing, staining, and identification supplies
11. Turbidity Meter to measure a 1.0 McFarland Standard
12. Sterile swabs

13. Recommended supplement (such as human blood) that will support the growth of fastidious organisms such as *Haemophilus spp*. and *Neisseria spp*. (due to absence of patient's blood in sample).

14. Organisms for testing. Examples of ATCC strains of common clinical iso-isolates recovered from blood are:

E. coli ATCC 25922	*S. aureus* ATCC 25923
S. pyogenes ATCC 19615	*E. faecalis* ATCC 29212
P. stuartii ATCC 33672	*S. maltophilia* ATCC 13637
H. influenzae ATCC 19418	*N. meningitidis* ATCC 13090
P. aeruginosa ATCC 27853	*K. pneumoniae* ATCC 33495

IV. QUALITY CONTROL

See Manufacturer's Package Insert and/or Operator's Manual for quality control requirements for the media and the instruments.

V. PROCEDURE

IMPORTANT NOTE: Please be sure to follow Good Laboratory Practices and Universal Precautions at all times during the test procedure. All materials should be disposed of properly as required by your institution.

For most bacterial strains*, a McFarland 1.0 Standard is equivalent to ~3 x 10^8 CFU/mL. Therefore, for most bacteria, a dilution of 10^6 (or three 1:100 dilutions) should result in a CFU of ~ 3 x 10^2 or ~3 x 10 CFU/0.1 mL inoculum (30 CFU).

1. Grow each organism to be tested overnight on the appropriate plated medium. Carefully examine and check each organism for purity. If there is a contaminant or the colonies have an unusual appearance, perform a Gram stain and/or identification to confirm the identity. If questions remain, do not use that organism at that time. Re-culture and repeat culture.

*NOTE: These dilutions are a guide and may need to be changed depending on the organism used to obtain the 10 to 100 CFU inoculum range. In the event that the colony count is below 10 or above 100, the experiment should be repeated with an appropriately adjusted dilution.

2. Add 10 mL of Normal saline to 4 test tubes labeled "A", #1, #2 and #3. Note the organism name to be tested on each tube. Additional tubes should be labeled for each organism to be tested as indicated above.

3. Begin with the test tube labeled “A”. Take a sterile swab and touch a few well isolated colonies. Place the swab into the tube of saline and swirl. With the colonies added, the turbidity meter should measure approximately 1.0 McFarland. If the meter does not read in this area, either: a) add more colonies if the reading is < 1.0; or b) dilute with sterile saline if the reading is > 1.0 to achieve the target 1.0 McFarland reading.

4. For a 10^6 dilution, add 0.1 mL of the standardized McFarland 1 organism suspension to the test tube labeled #1. Mix well.

5. For a 10^4 dilution, add 0.1 mL of the test tube #1 organism suspension to the test tube labeled #2. Mix well.

6. For a 10^2 dilution, add 0.1 mL of the test tube #2 organism suspension to the test labeled #3. Mix well. Tube #3 will be the inoculum used for the test and reference blood culture vials.

7. To simulate clinical blood culture specimens, human blood should be added to each culture vial. Wipe the tops of the blood culture vials with a 70% isopropyl pad. Add 3 to 10 mL of blood [or appropriate blood volume for the media type(s)] to each test and reference blood culture vial prior to organism inoculation.

8. Plate Count - Prepare an agar plate of the final inoculum using the agar medium appropriate for each organism. Take 0.1 mL inoculum of tube #3 and inoculate the plate. Use the “spread plate” or similar sterile technique to evenly distribute the inoculum.

9. Wipe the tops of the vials with a 70% isopropyl alcohol pad. Label the test and reference blood culture vials with the organism’s name. Inoculate each test and reference blood culture vial (duplicate or triplicate if desired) with 0.1 mL of tube #3 of that organism.

10. Incubate both the inoculated test and reference blood culture vials in their respective instrument (or incubator) as directed in the package insert(s) or laboratory procedure manual. Incubate the plate count media at 35^oC or as directed by recommended laboratory procedures.

11. Accurately record all results for both the test and reference blood culture systems used (i.e. organism CFU per vial, time to detection, plots, Gram stain/subculture/identification results, etc.).

12. Evaluate data generated according to laboratory method validation protocol.

RECOMMENDATIONS:

1. The test medium and the reference medium should be inoculated with bacteria at the same time.

2. Uninoculated sterility control vials and vials with added supplement (human blood)/no organism, of both the test and reference blood culture vials, should be included.

3. If possible, fresh human blood (3 to 10 mL) should be added to simulate clinical conditions. In most cases, optimal growth and detection occurs when the maximum blood volume for each medium type is used.

VI. LIMITATIONS

Care must be taken to prevent contamination of the vials during inoculation with the organism suspension.

In the event that the colony count is below 10 or above 100, the experiment should be repeated with an appropriately adjusted dilution.

VII. REFERENCES

Seeded Blood Culture Evaluation. Document MA-0100. Becton Dickinson Microbiology Systems

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