| KCMC Biotechnology Laboratory, Microbiology | STANDARD OPERATING PROCEDURE | Effective Date 30 May 2006 | SOP-Number MIC.010 |
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**Title: ISOLATOR BLOOD CULTURES FOR ISOLATION OF MYCOBACTERIA**

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**Document History:**

| Version Number | Reason for Changes | Date |
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**Title: ISOLATOR BLOOD CULTURES FOR ISOLATION OF MYCOBACTERIA**

**PURPOSE**

Procedures for the processing of Isolator tubes primarily for isolation of mycobacteria from blood and calculating the number of CFUs/ml of blood. Other organisms (e.g. bacteria and fungi) may also grow on the medium.

**PRINCIPLE**

The Isolator tube contains agents that lyse the cellular components of the blood, block coagulation, and cushion components during centrifugation. A 5.0 ml volume of blood is drawn into the tube, mixed and centrifuged to sediment microorganisms. The supernate is removed and the sediment planted on 2 Middlebrook 7H10 agar plates. The number of colony forming units (CFUs) per ml of blood is calculated from the number of colonies present on the plates and original blood volume.

**SCOPE**

This Standard Operating Procedure applies to all microbiology personnel who are trained and competent in processing samples collected in Isolator tubes.

**PRECAUTIONS**

Use standard precautions and appropriate protective equipment (gloves) when handling patient samples to protect against bloodborne pathogen exposure.

**SPECIMEN COLLECTION/TRANSPORT**

Refer to SOP MIC.012 SPECIMEN COLLECTION FOR ISAAC STUDIES for blood collection and transport procedures.

Collection tube: Isolator 10 Lysis Centrifugation tube (Wampole Laboratories)
Blood Volume: 5.0 ml

**SPECIMEN RECEIPT**

Record volume of blood collected on BLOOD CULTURE RESULT Teleform.

All Isolator tubes containing blood will be processed.

**SPECIMEN PROCESSING**

**Materials:**

ISOSTAT Cap
ISOSTAT Press
ISOSTAT Supernate pipet.
ISOSTAT Concentrate pipet
Middlebrook 7H10 Agar plates (2) labeled with study ID#, current date and date in 42 days.

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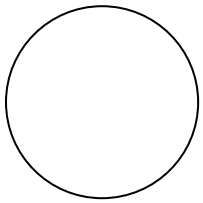
**Procedure:**

1. Spin Isolator tube for 30 min at 4000 rpm.
2. Clean top of Isolator tube with 70% alcohol and let dry.
3. Place ISOSTAT CAP over top of stopper while grasping only the sides of the cap.
4. Position cap under press apparatus.
5. Pull down on handle and release to pierce a hole in top of tube.

   ***Perform remainder of steps under biohazard hood:***

6. Remove all the supernate using the ISOSTAT supernatant pipette leaving the concentrate at the bottom of the tube.
7. Mix concentrate for 10 seconds using a vortex mixer at the highest setting.
8. Collapse COMPLETELY the bulb of the ISOSTAT CONCENTRATE PIPET and insert into tube. Withdraw all concentrate.
9. Dispense concentrate in a straight line down surface of each of 2 Middlebrook 7H10 agar plates (avoid edge of plate).
10. Using the tip of the pipette streak with 15-20 perpendicular passes through inoculum as shown in the diagram below.



11. Record the date and time plates were inoculated on the BLOOD CULTURE RESULTS Teleform.
12. Place plates in plastic bag and incubate at $35^{o}$ C in 5% $CO_2$.

**EXAMINATION OF CULTURES**

1. Examine plates for growth daily for 6 weeks using a magnifying lamp.

**POSITIVE CULTURE WORKUP**

1. Record the following on the BLOOD CULTURE RESULT Teleform:

Date and time growth was first detected (Time To Positive in hours from when the specimen was planted).

2. Calculate the number of CFUs/ml of blood as follows:

   a. Continue to incubate plates an additional week to assure mature growth.

   b. Count the number of CFUs on all plates.

   c. Calculate number of Colony forming Units (CFUs) per ml using the following formula:

$$\frac{\text{Total number of colonies on both plates}}{\text{Number of plates on which organism would be expected to grow}} \times \frac{\text{Number of plates inoculated}}{\text{Blood Volume}}$$

   d. Record number of CFUs on BLOOD CULTURE RESULT Teleform.

   e. Make a smear from the colonies and stain with Kinyoun. Refer to MIC.004 Kinyoun Stain.

   f. If Kinyoun stain is negative, prepare another smear and stain with Gram stain. Refer to MIC.021 Gram Stain Procedure.

**AFB WORKUP**

1. When there is at least one loopful of growth available, organism may be tested to identify *M. tuberculosis*. Refer to MIC.037 Accuprobe Culture Identification for M. tuberculosis SOP. Place plate in rack in incubator labeled “For GenProbe Testing”.
2. Subculture mycobacteria to a Lowenstein Jensen (LJ) medium slant to obtain additional growth and sufficient inoculum for archiving. Incubate LJ at $35^{o}$ C until good growth is obtained.

**NON-AFB WORKUP**

1. Proceed with subculture and identification of organisms based on colonial and microscopic morphology.

**REFERENCES**:

Agy, MB, etal. 1989. Evaluation of 4 mycobacterial blood culture media: BACTEC 13A, Isolator/BACTEC 12B, Isolator/Middlebrook agar, and a biphasic medium. Diag. Microbiol;. Infect. Dis. 12:308.

Witebsky, FG, etal. 1988. Comparison of BACTEC 13A medium and DuPont Isolator for detection of mycobacteraemia. J. Clin. Micro.26:1501.

**APPENDIX – ISOLATOR PROCESSING DIAGRAMS**