KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 24 May 2007	SOP-Number MIC.015
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Title: PROCESSING OF SPUTUM AND GASTRIC ASPIRATE SPECIMENS FOR MYCOBACTERIAL CULTURE

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Document History:

Version Number	Reason for Changes	Date

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PURPOSE

Procedures for inoculation of culture media and smear preparation with non-blood specimens for mycobacterial culture.

PRINCIPLE

Digested sputum and gastric acid specimens, and CSF are inocolated on 1 Lowenstein-Jensen Medium, slant (LJ), 1 Middlebrook 7H10 Agar plate and 1 MP BacT/ALERT culture bottle to which has been added appropriate supplements (MB BacT Antibiotic Supplement and/or MB BacT Reconstitution Fluid).Smears are prepared for Auramine fluorescent acid-fast stain.

SAFETY

- Observe standard precautions when handling patient specimens to avoid exposure to bloodborne pathogens. Refer to SAF.001 BIOHAZARD SAFETY SOP.
- All specimen processing must be done in the biohazard safety hood and appropriate personal protection worn (gown, gloves, particulate respirator mask).
- Use care when handling unstained slides to avoid touching infectious material.

SPECIMENS

- Sediment from digested sputum or gastric aspirate (Refer to MIB.007 SOP for specimen digestion/decontamination procedures.)
- CSF – centrifuged at 3500 rpm for 30 minutes and supernate removed.

MATERIALS:

Disposable sterile inoculation loops
Disposable 1 ml Pasteur pipettes
5 mL syringe with 18 g needle per sample
1 ml tuberculin syringe per sample
70% alcohol wipes
Clean glass slides (1 per sample)
Slide warmer set at position 7 (= 70°C)
BacT/ALERT instrument

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Media & Supplements:

1 Lowenstein-Jensen (L-J) slant* (store at 2-8^{o} C)
1 Middlebrook 7H10 agar plate* (store at 2-8^{o} C)
1 MP BacT/ALERT bottle* (store at 2-8^{o} C)
*For each sample – warm to room temperature

MB BacT Antibiotic Supplement (store at 2-8^{o} C)
- Amphotericin B
- Azlocillin
- Naladixic acid
- Polymyxin B
- Trimethoprim
- Vancomycin

MB BacT Reconstitution Fluid (store at 2-8^{o} C)
- Oleic acid
- Glycerin
- Amaranth
- Bovine serum albumin

PREPARATION OF SUPPLEMENTS FOR MP BOTTLE

Supplements will be used as follows:

Digested sputum and gastric aspirates – 0.5 ml Antibiotic supplement which has been reconstituted with Reconstitution Fluid.

CSF – 0.5 ml of Reconstitution fluid only.

Reconstitution of Antibiotic supplement:

1. Remove a vial of Antibiotic Supplement and a vial of Reconstitution Fluid from the refrigerator. (If a vial of antibiotic supplement has already been reconstituted this may be used if within 7 days of reconstitution)
2. Add 10 ml of Reconstitution Fluid to the Antibiotic Supplement and swirl gently to dissolve.
3. Mark the date of reconstitution and the expiration date 7 days from reconstitution date. Discard unused Antibiotic Supplement after 7 days.
4. Return vials to the refrigerator.

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CULTURE INOCULATION AND SMEAR PREPARATION:

1. Label the following with the patient's PID# and Date of processing; On the LJ slant and Middlebrook plate enter the date 6 weeks from date of inoculation:
   - 1 LJ Slant
   - 1 Middlebrook 7H10 Agar plate
   - 1 MP BacT/ALERT bottle
   - 1 Glass slide
2. Clean MP bottle septum with an alcohol wipe and let dry.
3. Add 0.5 ml of reconstituted Antibiotic Supplement to each MP bottle that will be inoculated with digested sputum or gastric aspirate.
4. Add 0.5 ml of Reconstitution fluid ONLY to each MP that will be inoculated with CSF.
5. Using a sterile disposable pipette, place a drop of the sediment onto a Lowenstein-Jensen slant and spread over the surface of the slant using the end of the pipette.
6. Place a drop of sediment on the 7H10 agar plate and streak for isolation with a loop.
7. Place a drop of the sediment onto a clean glass slide near the frosted end with a sterile disposable pipette and smear it thinly in a circle about 1.5 cm in diameter using the pipette . Place slide on slide warmer for 2 hours to dry and fix.
8. Using a 5mL syringe with 18G needle, aspirate 0.5 mL of specimen and inoculate an MP bottle. Disinfect bottle septum with _____
9. When processing is complete and specimens, smears and cultures have been removed, spray hood surface liberally with disinfectant and let stand 10 minutes before wiping dry. Discard towels in biohazard waste container.
10. Load MP bottle in BacT/ALERT instrument.
11. Incubate LJ and Middlebrook agars as follows:

MEDIUM	INCUBATION CONDITIONS	INCUBATION TIME
LJ Slant	35^{o} C, Aerobic incubator	6 weeks
Middlebrook 7H10 Agar	35^{o} C, CO2 Incubator in sealed plastic bag.	6 weeks

12. Stain the slide with Auramine-Rhodamine fluorescent acid-fast stain. (Refer to SOP MIC.005 AURAMINE STAIN)

REFERENCES

Package insert, BacT/ALERT MP, bioMerieux, Inc., Durham, NC, December 2004

Pfyffer, GE, etal. 2003. *Mycobacterium:* General Characteristics, Isolation and Staining Procedures. In: Manual of Clinical Microbiology, 8th Ed. Murray, PR, etal (eds), ASM Press, Washington, DC.