TITLE BODY FLUIDS, SOLID TISSUE AND BONE MARROW CULTURES

PRINCIPLE / PURPOSE: Microorganisms encountered in solid tissue, bone, bone marrow and sterile fluids (i.e. pleural, peritoneal, pericardial, joint, dialysis, etc.) may be bacterial, fungal, viral or parasitic. In response to infection, fluid may accumulate in any body cavity. Specimens are collected by percutaneous needle aspiration or needle biopsy or dialysis procedures.

SCOPE: This procedure applies to the setup and culture of body fluids, solid tissue and bone marrow specimens.

SPECIMEN:

Patient Preparation: Disinfect area prior to specimen collection.

Type: Specimens collected by percutaneous needle aspiration or needle biopsy or dialysis.

Minimum of 500 µL (0.5 mL) of body fluid.

Note: If ≤500 µL is received, notify the physician of modified processing; only a gram stain and chocolate plate will be set up. “Interpret results with caution due to limited specimen volume” must be documented in LIS.

Handling Conditions: Send aseptically collected specimen in an appropriate anaerobic container or sterile transport medium or blood culture bottles to the microbiology lab immediately.

EQUIPMENT AND MATERIALS:

Equipment:

Loop Sterile disposable pipette

Biological safety cabinet Centrifuge

35°C, CO2 incubator Sterile grinder

Vortex

Materials:

TSA blood agar – CO2

Chocolate agar – CO2

MacConkey agar – CO2

CDC – AnO2

Anaerobic Thioglycollate – AnO2

BacT/ALERT SA and SN Bottles- Aerobic and Anaerobic (Blood culture bottles)

Preparation:

All media are BBL and stored at 2-8° C. After plating, media are incubated at 35-37° C.

QUALITY CONTROL: Selected types of media are checked for sterility and performance using ATCC strains.

PROCEDURE - STEPWISE:

Use a biological safety cabinet when opening a culture container or potentially creating aerosols. Always wear gloves when handling a specimen.

A. Body Fluids – Received in Sterile Tube

1. Centrifuge the fluid for 10 minutes if the quantity of fluid is greater than 5 ml.
2. Aspirate the supernate using a sterile pipet, leaving approximately 0.5 to 1 ml of sediment. Save the supernatant for additional studies.
3. Vortex the sediment 30 seconds to resuspend the pellet.
4. Inoculate the media using 2 drops of the sediment.
5. Make and stain a Gram stain. Record stain results on the worksheet and in the LIS.
6. Incubate media according to Media section of this procedure.

B. Bone or Tissue

1. Remove mortar and pestle from sterile package. They are located in the top drawer near sink in setup area.
2. Process sample in Biological safety cabinet using PPE barrier.
3. If specimen is a large piece of tissue, then it may be necessary to cut the specimen into smaller pieces using sterile scissors or mince the specimen in the lid of a Petri dish with a sterile scapel.
4. Pour 1-2 ml of thio broth into grinder.
5. Place sample in sterile grinder and grind specimen using pestle.
6. Inoculate the emulsifed sample to culture media and make a smear for Gram stain.
7. Incubate media according to Media section of procedure.

C. Dialysis Fluid – Body Fluid – Received in BacT/ALERT SA/SN Bottles

1. Label bottles with barcode labels; including patient name, accession number, date, etc.
2. Positive cultures are to be removed from BacT/ALERT immediately.
3. Print “unload positive” report and graph.
4. Plate positive cultures on BAP, CHOC, MAC, AND CDC media.
5. Place aerobic media in CO2 incubator and anaerobic media in anaerobe box.
6. Make and stain a direct gram stain from the positive bottle.

Reading plates:

Day 1: Examine aerobic plates at 12-24 hours for potential pathogens.

Reincubate media.

Day 2: Examine aerobic and anaerobic plates for potential pathogens.

Day 3: Examine aerobic plates for potential pathogens.

Day 4: Examine aerobic plates for potential pathogens. Examine anaerobic

subcultures for potential pathogens using aerobic plates for comparison.

Finalize report.

Perform ID and AST (where appropriate for organism) on all isolates.

INTERPRETATION & REPORTING RESULTS:

Reporting Format:

Semiquantitate growth using the terms:

RARE – 1-2 colonies

LIGHT GROWTH – 1st quadrant

MODERATE GROWTH – 2nd quadrant

HEAVY GROWTH – final quadrant

For cultures that are negative use the following LIS code:

NGAA5 – No GROWTH AEROBICALLY OR ANAEROBICALLY IN 5 DAYS (If cultured via BacT/ALTERT SA/SN bottles

NGAA4 - No GROWTH AEROBICALLY OR ANAEROBICALLY IN 4 DAYS

If from BacT/ALERT SA or SN bottle do not quantitate.

PROCEDURE NOTES:

* ORGANISMS OF INTEREST:

BACTERIAL PATHOGENS ISOLATED FROM PLEURAL FLUID

Streptococcus pneumoniae

Staphylococcus aureus

Haemophilus influenzae

Enterobacteriaceae

Pseudomonads

Anaerobes- secondary to aspiration pneumonia or lung abscesses other organisms associated with pneumonia other streptococci, Nocardia sp., Actinomyces sp.

BACTERIAL PATHOGENS ISOLATED FROM PERITONEAL FLUID

ORGANISMS IN PRIMARY PERITONITIS

Streptococcus pneumoniae

Group A streptococcus

Enterobacteriaceae

Pseudomonads

Staphylococci

Neisseria gonorrhoeae

ORGANISMS IN SECONDARY PERITONITIS

Neisseria gonorrhoeae

Anaerobes

Enterococci

Other streptococci

Enterobacteriaceae

Bowel flora

BACTERIAL PATHOGENS ISOLATED FROM PERITIONEAL DIALYSIS FLUID

Staphylococcus epidermidis

Staphylococcus aureus

Streptococci

Aerobic and faculative Gram-negative rods

Candida sp.

Corynebacterium sp.

BACTERIAL PATHOGENS ISOLATED FROM JOINT FLUID

Staphylococcus aureus

N. gonorrhoeae

H. influenzae (< 2 years old)

Streptococci

Bacteroides sp.

Fusobacterium necrophorum

BACTERIAL PATHOGENS ISOLATED FROM BONE

Staphylococcus aureus

Salmonella sp.

Haemophilus sp.

Enterobacteriaceae

Pseudomonas sp.

Anaerobes

Yeast

* All plates are saved for 6 additional days at room temperature in the storage location.
* Some organisms may require special media or extended incubation in order to be isolated (ex. Actinomyces, Brucella, Legionella). The physician has to notify the lab when these types of organisms are suspected for appropriate handling of the culture.

LIMITATIONS OF THE PROCEDURE: Failure to adequately process fluid tissue or bone marrow specimens could result in inappropriate patient care.

REFERENCES:

ASM: Clinical Microbiology Procedures Handbook, ASM, sec 3.5, 2008.

Finegold, S.M. and W.J. Martin, “Diagnostic Microbiology”, 6th ed. C.V. Mosby Co., St. Louis, pp314-328, 1982.

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Bailey and Scott’s “Diagnostic Microbiology”, 8th edition pp. 49-64, 81-100, 279-289.

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HISTORY PAGE

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Written By: Leslie Benfield/Jacee Farmer

Manual in which Hard Copy of this SOP is located: Microbiology Manual IV

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Supersedes Procedure:

SOP CHANGE CONTROL

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