TITLE: Sterile Body Fluid Cultures (Excluding Blood, CSF, and Urine)

PRINCIPLE / PURPOSE: Infection of normally sterile body fluids often results in severe morbidity and mortality, therefore, rapid and accurate assessment of these samples is extremely important. Any microorganism isolated where there is not “normal flora” must be considered potentially significant. Use of prostheses, immunosuppressive therapy and long-term care of individuals with chronic debilitating diseases has an increased likelihood of this type of infection. Proper evaluation assumes that proper collection and transport techniques are utilized such that detected organisms are not contaminants. All isolates must be reported. The physician in consultation with a microbiologist can make final interpretation if necessary.

SCOPE: This procedure applies to the set up and reporting of fluid specimens, excluding blood, CSF, and urine.

Complexity: High

Safety:

* The required personal protective equipment for this procedure:
	+ Gloves; optional
	+ Approved lab coat, worn closed
	+ Biological Safety Cabinet
* Gloves and lab coats should be worn at all times during analysis of the samples
* Raw samples must be opened and dispensed within a bio-safety cabinet or behind safety shield
* Due to potential hazards no sniffing of plates

SPECIMEN:

* Joint or synovial fluid
* Pleural fluid: thoracentesis, empyema
* Peritoneal fluid: ascites, paracentesis, dialysis
* Pericardial fluid
* Culdocentesis fluid
* Amniotic fluid: amniocentesis

 Handling Conditions:

 Send aseptically collected specimen in an appropriate sterile container to the microbiology lab in a timely manner.

Fluids may be transported to the laboratory in a sterile syringe (capped and needle removed) or other sterile tube/container.

Unacceptable Specimen:

 Fluids in citrate or EDTA tubes – Some organisms are inhibited by these anticoagulants.

EQUIPMENT AND MATERIALS:

Equipment:

Loops

Biological safety cabinet

 CO2 incubator

 BacT/Alert

Vortex

Sterile disposable pipette

Glass slides

Centrifuge

BAP blood agar

Chocolate agar

MacConkey agar

ABAP, KV, Pea

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

SAB and SABCC for fungal requests

CALIBRATION: BACT/Alert cells are calibrated as needed and once per year (preventative maintenance).

QUALITY CONTROL: Laboratories can accept the quality control of the manufacturers of exempt media in accordance to CLSI guidelines.

Refer to the Media Quality Control Procedure for non-exempt media.

PROCEDURE:

Cytocentrifuge and Inoculation Note: Specimen such as synovial fluids, amniotic fluid, and sometimes peritoneal and dialysis fluids may have a high protein and cell count, which can make a cytospin smear difficult to read. It may be necessary to try the cytospin preparation and gram stain to determine if this techniques is adequate. A smear prepared from the sediment after centrifugation might be a better option.

Sterile Fluid Specimens >2.5 mL:

1. Place 2 – 3 drops of non-centrifuged fluid into two cytofunnel kits.
2. Insert two cytospin slides labeled with the patient’s name, accession number, type of fluid and date. DO NOT use work card labels for the slides. Write the information in pencil.
3. Cytocentrifuge for 10 minutes at 1200 rpm (preset on cytospin).
4. Dry the slides under the biosafety cabinet. Slide may be heat fixed on the slide warmer.
5. Insert the non-centrifuged fluid aseptically into SA and SN BACT/Alert (blood culture) bottles.
* Thoroughly clean the top of each bottle’s rubber stopper with alcohol.
* Draw up the fluid using a sterile syringe and needle.
* Aseptically insert the fluid in to the bottle.
* Add equal amounts to each bottle (aerobic and anaerobic).
* 5 – 10 mL of fluid in each bottle is optimal.
* Each bottle must contain at least 1 mL of fluid.
* Do not inoculate bottle with more than 10 mL
1. Using the work card labels, label each bottle and load onto the BACT/Alert analyzer.
2. Gram stain and read cytospin slides. (Refer to Gram Stain and Cytospin procedures)

Sterile Fluid Specimens >0.5 but <2.5 mL:

1. Using a sterile pipette, place 2 – 3 drops of fluid into two cytofunnel kits.
2. Place 1 – 2 drops on each appropriately labeled media plate. (Refer to posted Setup Chart for media selection)
3. Streak plates for isolation (3 or 4 quadrants).
4. Place plates in the CO2 incubator labeled “Systemic”.
5. Insert two cytospin slides labeled with the patient’s name, accession number, type of fluid and date. DO NOT use work card labels for the slides. Write the information in pencil.
6. Cytocentrifuge for 10 minutes at 1200 rpm (preset on cytospin).
7. Dry the slides under the biosafety cabinet. Slide may be heat fixed on the slide warmer.
8. Gram stain and read cytospin slides. (Refer to Gram Stain and Cytospin procedures)

Sterile Fluid Specimens <0.5 mL:

1. Gram stain and read cytospin slides. (Refer to Gram Stain and Cytospin procedures)
2. Using a sterile pipette, place 1 drop of specimen on an appropriately labeled slide with the patient’s name, accession number, type of fluid and date. DO NOT use work card labels for the slides. Write the information in pencil.
3. Use a sterile loop to spread the drop over the center of the slide.
4. Using a sterile pipette, place 1 drop of fluid on each appropriately labeled media plate (Refer to posted Setup Chart for media selection).

Note: Specimens received with 1 or 2 drops of fluid should be plated on a chocolate plate ONLY. Free text comment “Interpret results with caution due to a limited specimen volume”.

1. Streak plate(s) for isolation.
2. Place plate(s) in CO2 incubator labeled “Systemic”.
3. Gram stain and read cytospin slides. (Refer to Gram Stain and Cytospin procedures)

PROCEDURE NOTES:

All body fluids must be processed as soon as possible.

Fluids that extremely turbid or thick will be plated directly and have gram stain slides prepared without centrifugation.

Fluids containing large clots should be centrifuged. Place the sediment containing the clotted material in a sterile tissue grinder. Add a small volume (<0.5 mL) of sterile nutrient both (thioglycollate) to the tissue grinder and gently homogenize the mixture to disperse the clots and release any trapped bacteria. Prepare slides for gram stain and inoculate media.

INTERPRETING AND REPORTING RESULTS:

Table 1

|  |  |  |  |
| --- | --- | --- | --- |
| Culture Observation | Consideration Factor | Actions/ID | Reporting |
| No Growth <18 hrs | Reincubate | Preliminary report | NGR (No growth, reincubated) |
| Growth <18 hrs | Scant growth, unable to determine | Preliminary report | TYTR (Too young to read) |
| Growth | Growth | Refer to Organism Workup Procedure | Refer to Organism Workup Procedure |
| No Growth 1 day | Reincubate | Preliminary report | NG1 |
| No Growth 2 days | Reincubate | Preliminary report | NG2 |
| No Growth 3 days | Reincubate | Preliminary report | NG3 |
| No Growth 4 days | Reincubate | Preliminary report | NG4 |
| No Growth 5 days(For BOTTLES only) | No growth | Final report | NGAA5 |

\*\*\*The micro automatic NO GROWTH key may also be used but enter all cultures with growth PRIOR to using this function.

Table 2

|  |  |
| --- | --- |
| Result/Keyboard Code | Interpretation |
| RG | RARE GROWTH |
| LGR | LIGHT GROWTH |
| MGR | MODERATE GROWTH |
| HG | HEAVY GROWTH |
| If from BacT/ALERT bottles do not quantitate |

Abnormal Results:

Infection Control and physician or RN are notified of the following multi-drug resistant organisms: (MDRO), CRE and KPC organisms.

The local health department must be notified with isolates of Neisseria gonorrhoeae, Neisseria meningitides, Haemophilus influenza, Listeria monocytogenes, and Streptococcus pyogenes.

Organisms Of Interest:

 NOTE: Any organism in pure culture may be considered a pathogen. Consultation

 with the physician may be warranted if questionable. (Refer to Responsible

 Microbiology Reporting)

 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

 Streptococcus pneumoniae

 Enterobacteriaceae

 Pseudomonas

 Staphylococci

 Neisseria gonorrhoeae

 Anaerobes

 Enterococci

 Streptococci

 Bowel flora

 BACTERIAL PATHOGENS ISOLATED FROM PERITIONEAL DIALYSIS FLUID

 Staphylococcus epidermidis

 Staphylococcus aureus

 Streptococci

 Aerobic and facultative 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

* Cultures containing 3 or more organisms may be contaminated.
* Coagulase Negative Staphylococcus, gram positive rods, and mixed anaerobes may be considered contaminates. Consultation with a physician may be warranted to determine whether or not these should be considered pathogens.
* Physician may notify the lab when Actinomyces, Brucella, or Legionella are suspected. Some organisms may require special media, extended incubation, or sent to a reference laboratory for isolation and identification.
* All plates are saved for 7 additional days 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.
* In culdocentesis specimens, there is the potential for the isolation of Neisseria gonorrhea

LIMITATIONS OF THE PROCEDURE: Failure to adequately process fluid could result in inappropriate patient care.

REFERENCES:

ASM: Clinical Microbiology Procedures Handbook, vol.1, 3rd Ed., ASM, Washington, 2010

ASM: Manual of Clinical Microbiology, vol. 1, 11th Ed., 2015.

Finegold, S. M. and W. J. Martin, “Diagnostic Microbiology”, 6th ed., C. V. Mosby Co., St. Louis, pp 70-73, 1982.

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

|  |  |  |  |
| --- | --- | --- | --- |
| Review Date | Signature | Mgmt. | Director |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |
|   |   |   |   |

SOP HISTORY PAGE

SOP Number: MICRO-733

SOP Title: Sterile Body Fluid Cultures

Written By: Jacee Farmer

Manual in which Hard Copy of this SOP is located:

Distribution:

Supersedes Procedure:

SOP CHANGE CONTROL

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | Approvals |   | Action | In |
| Mgmt. | Date |  Director | Date |   | Effect |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|  |  |  |  |  |  |
| Date archived: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |
| Reason: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Initials:\_\_\_\_\_\_\_\_\_\_ |  |
|  |  |  |  |  |  |