|  |  |  |  |
| --- | --- | --- | --- |
| **SOP Number:** | M.3.10 | **Effective Date:** | 04/2013 |
| **Department:** | Microbiology | **Revision Date:** |  |
| **Policy (P), Procedure (PR)or Both (P/P):** | PR | **Version:** | 1 |

|  |  |  |
| --- | --- | --- |
| Applicable Standards |  | Version History |
| Standard | Organization  |  | Version | Effective Date | Retired Date |
| Mic.22495 | CAP |  | 1 | 04/01/13 |  |
|  |  |  |  |  |  |
|  |   |  |  |  |  |
|  |   |  |  |  |  |
| Related Documents |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |  |
| Review History (Up to the Last 15 Occurrences) |
| Date | Version | Revision Type | Review By/Initials & Date |
| 03/20/13 | 1 | Director Review | J. Lewis M.D. |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |

|  |
| --- |
| Distribution |
| CSHCC Memorial Microbiology SOP |
| CSHCC Shoreline Microbiology SOP |
|  |
|  |
|  |
|  |
|  |

**PRINCIPLE:**

Infection of normally sterile body fluids often results in severe morbidity and mortality therefore, rapid and accurate microbiological assessment of these samples is important to successful patient management. Most organisms infecting these sites are not difficult to culture, but determining the significance of low numbers of commensal cutaneous microorganisms does present a challenge. With the increased use of prostheses, immunosuppressive therapeutic regimens, and longterm care of individuals with chronic

debilitating disease, the likelihood of true infection with commensal organisms has

increased, making accurate diagnoses difficult. Care must be taken during specimen collection and transport to ensure that the specimen is not contaminated.

Any microorganism found in a normally sterile site must be considered significant, and all isolates must be reported.

**SPECIMEN COLLECTION, TRANSPORT, AND HANDLING:**

Body fluid specimens collected by percutaneous aspiration for pleural, pericardial, peritoneal, amniotic, and synovial fluids

**Use care to avoid contamination with commensal microbiota.**

1. Clean the needle puncture site with alcohol, and disinfect it with an iodine solution (1 to 2% tincture of iodine or a 10% solution of povidone-iodine [1% free iodine]) to prevent introduction of specimen contamination or infection of patient. (If tincture of iodine is used, remove with 70% ethanol after the procedure to avoid burn.)
2. Aseptically perform percutaneous aspiration with syringe and needle to obtain pleural, pericardial, peritoneal, or synovial fluid. Use safety devices to protect from needle exposure.
3. Submit fluids via one of the following methods.
4. A sterile, gassed-out tube or a sterile blood collection tube without preservative (e.g., sterile Vacutainer brand red-top tube); however, fluids in such tubes may clot during transport. Alternatively, fluids may be placed in a purple-top tube containing an anticoagulant such as heparin or SPS, although some fastidious organisms may be inhibited if the specimen is delayed in transport. EDTA and citrate anticoagulants are the most likely to inhibit microorganisms in fluid specimens and should not be used.
5. Syringes that have been capped with a Luer-Lok (with needle removed) prior to transport may be accepted for culture provided the specimen has not clotted inside the syringe and there is no leakage during transport which could result in contamination of the culture. The laboratory may reject specimens that have clotted in a capped syringe because they cannot be processed for culture without inadvertently contaminating the specimen.

**Specimen transport**

1. Submit to laboratory as soon as possible.
2. Do not refrigerate.
3. Label specimens with patient demographics and date, time, and site of collection: e.g., left knee joint fluid.
4. Record the patient diagnosis for improved processing of specimen.

**Rejection criteria**

1. Collect prior to antimicrobial therapy for greatest diagnostic sensitivity.
2. Do not submit specimens from drains after they have been infused with antimicrobial agents.
3. **NOTE:** Swabs afford the least desirable sample for culture of body fluids and should be discouraged as devices for transport, since the quantity of sample may not be sufficient to ensure recovery of a small number of organisms.
4. Contact physician if specimen is insufficient for the number of tests requested. **NOTE:** Routine bacterial culture is sufficient for culture for *Candida* species, if blood culture bottles are used or specimen is centrifuged. Fungal cultures of joint and abdominal specimens are occasionally indicated (especially for *Blastomyces dermatitidis* and *Histoplasma capsulatum*) but should be discouraged routinely. AFB cultures should not be routine but should be limited to those with a clinical indication.
5. Invasively collected specimens in leaky containers must be processed, but alert the physician of the possibility of contamination.

**MEDIA:**

1. BAP
2. CNA
3. MAC
4. CHOC
5. Gram Stain – Cytospin on clear and non-viscous specimens

**PROCEDURE:**

**Inoculation**

1. Process specimen as soon as it is received within the Biological Safety Cabinet.
2. When specimen volumes allow, centrifuge specimen at 1500 G for 15 minutes and inoculate media with sediment.
3. If < 1ml is received do not spin but use unconcentrated specimen to setup plates and gram stain.
4. If only a few drops are received directly inoculate only the CHOC plate and perform Gram Stain as able. Note on report the volume received.
5. Inoculate anaerobic medium as appropriate with 2 or 3 drops of specimen. For pleural fluid or drainage fluid, perform anaerobe culture only on request and if specimen was transported anaerobically.
6. For peritoneal fluid contaminated with bowel contents, add media for anaerobic isolation of mixed anaerobic microbiota.
7. Prepare Gram stain by cytocentrifugation on clear aspirates and nonviscous body fluids. For viscous fluids prepare Gram stain by placing 1 or 2 drops of fluid specimen on an alcoholrinsed slide. Allow the drop(s) to form one large drop. Do not spread the fluid.
	1. Air dry the slide in a biosafety cabinet or covered on a slide warmer.
	2. Fix smear with methanol and stain (see procedure 3.2.1 for staining details).

**Incubation**

1. Incubate plates at 35 to 37°C. Chocolate plates should be put in 5-10% CO2.
2. Save some specimen at 4°C for 1 week for further testing.

**Gram stain**

1. Interpret Gram stains immediately.
	1. If positive and sterile site, notify the physician and or nursing unit immediately.
	2. *If mixed morphologies are seen in Gram stain, retrieve the specimen and inoculate selective anaerobic plates to select for mixed pathogens.*

**Culture examination**

1. Examine media for macroscopic evidence of growth at 24 h.
2. If no visible growth is observed on the culture media, reincubate for a total of 3 days. **NOTE:** For specific requests or where patient history or clinical state suggests a slow-growing pathogen (e.g., *Brucella*), additional incubation time is appropriate.
3. Cultures with growth require physician notification if sterile site.
4. Correlate culture results with those of the direct Gram stain.
5. Identify all organisms, using rapid tests as applicable.
6. Do not perform complete identification if the isolate is one or two colonies of a coagulase-negative staphylococcus on one plate medium with no growth in the broth.
7. For peritoneal specimens that contain mixed gastrointestinal microbiota and no predominant organism, generally group organisms into “enteric,” “anaerobic,” and “skin” microbiota and do not identify further. However, screen the culture for the usual fecal pathogens. Detection of yeasts, *S. aureus, Pseudomonas aeruginosa,* or, possibly, vancomycin-resistant enterococci may represent etiologies not covered by empiric regimens and should be listed individually. **NOTE:** Empiric antimicrobial therapies are selected for the treatment of gastrointestinal tract microbiota, including anaerobes, enteric gram negative bacilli, and enterococci. To attempt to isolate and report each of these agents is labor-intensive and does not add to the requirement to treat the patient with agents that are effective against all the usual microbiota.
8. Perform antimicrobial susceptibility testing as appropriate per CLSI standards.
9. Hold positive culture plates for at least 7 days in case further workup is desired.

**REPORTING RESULTS:**

1. When reporting negative results, indicate the incubation time in the report.
2. If a plate contaminant is suspected, add a notation: “Unable to differentiate contamination of plate media from true infection; suggest repeat culture of appropriately collected specimen.”
3. Report the probable genus and species as soon as preliminary tests are completed. For mixed abdominal microbiota, a general statement listing the groups of organisms may be sufficient, e.g., “Numerous enteric rods, numerous mixed anaerobic microbiota, including *Clostridium perfringens* and *Staphylococcus aureus,* present.”

**INTERPRETATION:**

1. Generally, a positive culture indicates infection with the organism.
2. WBCs are usually present with infections of body fluids.

**LIMITATIONS:**

1. False-positive cultures can result from contamination of the specimen with skin microbiota.
2. False-negative results can be caused by low numbers of organisms, prior antimicrobial treatment, or the fastidious nature of the infective organism.
3. Many organisms that cannot be easily cultured cause arthritis. These include *Borrelia burgdorferi,* the agent of Lyme disease.

**REFERENCES:**

1. Clinical Microbiology Procedures Handbook. 3.5. Body Fluid Cultures. March 2007 Update.
2. CAP Checklist - Microbiology. 09.25.2012. MIC.22495 Centrifugation of Body Fluids.