**I. 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.

Culture of the specimen should include the most likely organisms to cause infection.

Joint infections are commonly caused by *Staphylococcus aureus,* but *Streptococcus pyogenes, Neisseria gonorrhoeae,* anaerobes, *Kingella kingae,* and *Brucella*

spp. may be likely causes of infectious arthritis. Automated blood culture systems

have been shown to be faster than lysiscentrifugation and have a higher yield. Pediatric bottles have the advantage of containing less sodium polyanethol sulfonate (SPS), which inhibits some organisms. For prostheticjoint infections, the importance of preoperative joint fluid cultures cannot be overemphasized. The diagnosis is increased by culture of large volumes, by culturing anaerobically, and by culturing more than one specimen. Typically the Gram stain shows inflammation, without the presence of bacteria.

Peritoneal fluid can be contaminated with numerous mixed gastrointestinal microbiota in cases of ruptured intestine, but in patients with chronic ambulatory peritoneal dialysis (CAPD) or spontaneous bacterial peritonitis (SBP), the likely pathogen is usually present in very low numbers. In CAPD the pathogens are staphylococci; viridans group streptococci; non-glucose-fermenting, gram-negative rods; *Candida albicans* and other *Candida* spp.; and fungi . Numerous studies have shown that culture of large volumes of fluid in blood culture bottles, rather than concentration by centrifugation, will result in a higher yield. Blood culture bottles are superior to the lysis-centrifugation system for diagnosis

of SBP.

**SPECIMEN COLLECTION, TRANSPORT, AND HANDLING**

*Refer to Table 3.5–1 for commonly submitted body fluids and synonyms.* **NOTE:** Because the body may respond to infections with infiltration of fluid, some of these sites may have fluid accumulation only during infection.

Specimen collection: body fluid specimens collected by percutaneous aspiration for pleural, pericardial, peritoneal, amniotic, and synovial fluids **NOTE:** Refer to procedure 3.3.1 for additional details. The following can be copied for preparation of a specimen collection instruction manual for physicians and other caregivers. It is the responsibility of the laboratory director or designee to educate physicians and caregivers on proper specimen collection.

**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. Immediately place a portion of the joint fluid or peritoneal fluid collected from patients with CAPD or SBP into aerobic and anaerobic blood culture bottles, retaining some (0.5 ml) in syringe for Gram stain and direct plating. Use the minimum and maximum volumes recommended by the bottle manufacturer (generally up to 10 ml is the maximum for each bottle). Alternatively, inoculate the blood culture bottles after receipt in the laboratory.
4. Submit other fluids and the remainder of specimens placed in blood culture bottles in one of the following
5. 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.
6. Anaerobic transport vial (for small-volume specimens)
7. 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. If only blood culture bottles are received, a Gram stain cannot be performed.
2. Collect prior to antimicrobial therapy for greatest diagnostic sensitivity.
3. Do not submit specimens from drains after they have been infused with antimicrobial agents.
4. **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.
5. 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.
6. 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

**Inoculation**

1. Process specimen as soon as it is received within the Biological Safety Cabinet.
2. Inoculate media with 2 or 3 drops of specimen.
3. If little specimen is received (1 or 2 drops), inoculate only CHOC and rinse tube with broth culture medium. Omit Gram stain. Note on report the volume received.
4. For greater than 2 ml of pericardial, peritoneal, amniotic, or synovial fluid specimens not received in blood culture bottles, inoculate aerobic and, if there is sufficient volume of specimen, anaerobic blood culture bottles. Do not add less than the amount recommended by the manufacturer, as the excess SPS may be inhibitory to growth of the infecting organism.
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 placing 1 or 2 drops of fluid specimen on an alcohol-rinsed slide.
8. Allow the drop(s) to form one large drop. Do not spread the fluid.
9. Air dry the slide in a biosafety cabinet or covered on a slide warmer.
10. Fix smear with methanol and stain (see procedure 3.2.1 for staining details).

**NOTE:** The use of a cytocentrifuge for preparation of the Gram stain from clear aspirates or nonviscous body fluids is highly recommended.

**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 aerobic (e.g., MAC and CNA) and anaerobic plates to select for mixed pathogens.*

**Culture examination**

1. Examine all plated and broth 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.

**c.** Incubate blood culture bottles for 5 to 7 days. Extend incubation if *Brucella*

culture is requested.

**3.** Cultures with growth

**a.** Notify physician of positive culture findings.

**b.** Correlate culture results with those of the direct Gram stain.

**c.** Identify all organisms, using the rapid tests listed in Table 3.3.2–5. For less common pathogens refer to procedures 3.18.1 and 3.18.2. **d.** Do not perform complete identification if the physician indicates that the organism is a probable contaminant or that the isolate is one or two colonies of a coagulase-negative staphylococcus on one plate medium with no growth in the broth.

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 (*see* procedures 3.8.1 and 3.8.2). 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.

Perform antimicrobial susceptibility testing as appropriate per CLSI standards.

Hold positive culture plates for at least 7 days.

**REPORTING RESULTS**

1. Refer to procedure 3.3.2 for general reporting procedure.
2. When reporting negative results, indicate the incubation time in the report.
3. 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.”
4. 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.”
5. Document notification of positive findings.

**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.