**Purpose**

To culture a body fluid specimen for growth of pathogenic organisms

**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. 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. For joint infections, the use of blood culture bottles produces faster results and a higher yield. For prosthetic joint infections, the diagnosis is increased by culture of large volumes, by culturing anaerobically, and by culturing more than one specimen.

Peritoneal fluid can be contaminated with numerous mixed gastrointestinal microbiota in cases of ruptured intestine. In patients with chronic ambulatory peritoneal dialysis (CAPD) or spontaneous

bacterial peritonitis (SBP), the likely pathogen is usually present in very low numbers. Culture of large volumes of fluid in blood culture bottles, rather than concentration by centrifugation, results in a higher yield.

**Acceptable Specimens**

* A sterile, red-top Vacutainer tube or green-top tube (heparin)
* Port-a-Cul anaerobic transport vial (for small-volume specimens)
* Capped syringe (needle removed)
* Sterile container
* Fluid in Blood Culture bottles with extra fluid submitted for plating.

**Rejection Criteria**

* If only blood culture bottles are received, a Gram stain cannot be performed.
* Call physician when fluid specimens are received on a swab. Swabs afford the least desirable

 sample for culture of body fluids. The quantity of sample may not be sufficient to ensure recovery

of a small number of organisms.

* 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 but should be discouraged routinely. AFB cultures should be limited to

those with a clinical indication.

* Invasively collected specimens in leaky containers must be processed, but alert the physician of the

 possibility of contamination.

* Specimens not received in a sterile container are rejected.
* Unlabeled or incorrectly labeled specimens are never acceptable.

**Specimen Transport**

* Submit to laboratory as soon as possible
* Do not refrigerate.

**Materials**

Blood culture bottles – Aerobic, Anaerobic and/or Peds

 5% Sheep blood agar – SBA

 Chocolate agar - CHOC

 CNA agar

 MacConkey agar - MAC

 Thio broth (Thioglycollate)

 For Anaerobic cultures – Brucella, PEA, and KV/BBE agars

 Gram stain reagents

 Biochemical test reagents

 Cytocentrifuge and holders

 Cleaned glass slides

 Sterile pipettes

 35⁰C CO2 incubator

**Quality Control**

 1. Verify that media meet expiration date and QC parameters

 2. See individual tests for biochemical test QC

**Procedure**

 **A. Inoculation** for aerobic and anaerobic culture **(if requested)**

1. Process specimen as soon as it is received. Check to see if two identifiers on the specimen and plate

 labels match.

 **NOTE**: Use of biosafety cabinet will avoid contamination of the culture or specimen as well as

 protect laboratory processing personnel.

 2. Record volume of specimen if less than 1mL on plate label.

 3. Place media labels on the appropriate plate.

 4. For specimens >1.5mL of pericardial, peritoneal, amniotic, or synovial fluid, inoculate aerobic and, if

 there is sufficient volume of specimen, anaerobic blood culture bottles.

* Use a Peds bottle for 1-3mL of fluid and adult aerobic and/or anaerobic bottles if the volume is 3 to 20mL.
* Save enough fluid to inoculate media plates.

 5. For specimens >1.5mL, that are not inoculated into a blood culture bottle:

 Centrifuge 15 minutes at 3500 rpm

 Using a sterile pipette remove the supernatant and place in sterile tube

 Use pellet at bottom of tube for culture.

 6. Inoculate BAP, CHOC, CNA and MAC with 2 or 3 drops of specimen.

* If little specimen is received (1 or 2 drops), inoculate only CHOC and rinse tube or syringe with Thio broth culture medium. Omit Gram stain. Note on report the volume received.
* If 0.5 to 1.5mL of fluid is received, inoculate media plates, make a gram stain slide, and inoculate the rest of the specimen into the THIO broth.

 7. If anaerobic culture is ordered, inoculate anaerobic plates with 2 or 3 drops of specimen.

 8. Dispersion of clots

 a. Pour the sediment containing clotted material into a sterile tissue grinder.

 b. Add a small volume (<0.5 ml) of sterile broth to the grinder, and gently homogenize this

 mixture to disperse the clots and release any trapped bacteria.

 **Note**: Do not grind the clots if a fungal culture is also requested for the sample. Tease the clots apart.

 vigorous grinding can kill hyphal filaments

 **B.** **Prepare Gram stain** by placing 1 or 2 drops of fluid specimen on an clean slide

 l. Allow the drop(s) to form one large drop. Do not spread the fluid.

 2. Air dry the slide in a biosafety cabinet or covered on a slide warmer.

 3. Fix smear with methanol and stain (see Gram Stain procedure for staining details).

 **Note**: Use of a cytocentrifuge for preparation of the Gram stain from clear aspirates or nonviscous

 body fluids is highly recommended (20).

* All clear fluids should be processed using the Stat Spin Cytofuge procedure
* Specimens with a low cell count should be pre-concentrated by centrifugation for 15 minutes at 3500 rpm
* The supernatant is removed using a sterile pipette, leaving approximately 0.5cc for culture and the Cytofuge gram stain
* Mix the pellet with the sterile pipette and place one to two drops of CSF in the Cytofuge concentrator
* The StatSpin Cytofuge settings used for Gram Stains are:

 Time: 10 minutes

 Speed: 44 (4400 rpm)

 **C. Incubation**

 1. Incubate plates at 34 to 37⁰C in 5% CO2. Alternatively, use a CO2-generating

 system to provide the proper atmosphere if a Co, incubator is unavailable.

 2. Incubate broths at 34 to 37⁰C in ambient air(non-CO2 incubator).

 **D. Save some specimen** at 4"C for I week for further testing.

 **E. Gram stain**

 1. Interpret STAT Gram stains immediately. All other ordered gram stains are read as soon as possible.

 Gram stains received before 2 pm are to be read before the end of the shift.

 2. If positive, notify the physician immediately.

 **F. 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.

 a. Read aerobic plates daily for 3 days for invasively collected specimens and 2 days for

 drainage cultures.

 **Note**: For specific requests or where patient history or clinical state suggests a slow-growing

pathogen (e.g., Brucella), additional incubation time is appropriate.

 b. For broth cultures, observe daily for growth. If growth is observed subculture, Gram stain

 if no growth on primary plates.

 c. Incubate blood culture bottles for 5 to 7 days. Extend incubation if requested by patient care

 provider.

 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 available.

 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 or diphtheriods on one plate medium with no growth in the broth.

 e. For peritoneal specimens that contain mixed gastrointestinal flora and no predominant

 organism, generally group organisms into "enteric mixed gram negative or mixed gram

 negative and positive flora", "anaerobic," and "skin" flora and do not identify further.

 Detection of yeasts, S. aureus, Pseudomonas aeruginosa, or possibly, vancomycin-resistant

 enterococci may represent organisms not covered by empiric regimens and should be listed

 individually.

 **NOTE**: Empirical antimicrobial therapies are selected for the treatment of normal

 gastrointestinal tract flora, including anaerobes, enteric gram-negative bacilli, and

 enterococci.

 f. Perform antimicrobial susceptibility testing as appropriate per CLSI standards.

 g. Hold positive culture plates or tubes for at least 7 days.

 h. Isolates from broth cultures may be contaminants. Work up of isolates should be directed

 by the clinical picture after consultation with the physician.

**Reporting Results**

A. Record enumeration, growth and colony morphology under each media type (analyte).

 B. When reporting negative results, indicate the incubation time in the report.

 C. If a plate contaminant is suspected, add a notation; **PRC** – “Probable contamination” and **RSPEC** –

 “Recollect specimen if clinically indicated” (if recollection is advisable).

 D. Report the probable genus and species as an “Isolate” as soon as preliminary tests are completed.

 For mixed abdominal flora, a general statement listing the groups of organisms may be sufficient.

 E. Document notification of positive findings.

 F. For low volume specimens <1mL add the comment “Culture results may be compromised due to the

 limited volume of specimen received.”-**LOVOL** and **LT1 “**Less than 1cc of specimen received”.

**Interpretation**

A. Generally, a positive culture indicates infection with the organism.

B. WBCs are usually present with infections of body fluids.

**Limitations**

 A. False-positive cultures can result from contamination of the specimen with skin flora

 B. False-negative results can be caused by low numbers of organisms, prior antimicrobial treatment, or

 the fastidious nature of the infective organism.

 C. Many organisms that cannot be easily cultured cause arthritis. These include Borrelia burgdo(eri,

agent of Lyme disease

**References**

Garcia, Lynne S. ed. Clinical Microbiology Procedures Handbook, 3rd Ed. 2007, ASM Press Washington,

DC. Vol. 1, pp. 3.5.1-3.5.7

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Date:** | **Author:** | **Date:** | **Validated by:** | **Date:** | **Approved by:** |
| 05/15/2014 | Elaine Krueger |  |  |  |
|  |
| **Annual Review** |
| **Date:** |  |  |  |  |  |  |  |  |  |  |  |  |
| **Initials:** |  |  |  |  |  |  |  |  |  |  |  |  |
| **Date:** | **Initials:** | **Revision and Reason** |
| 5/15/2014 | EK | Reformatted and revised – more complete - low volume reporting added  |
|  |  |  |