**MICRO.CULT.5.0 STERILE FLUID AND TISSUE CULTURE**

**PRINCIPLE**

Sterile body fluids and tissues are examples of sites from which any growth of organisms will be worked up. These specimens are generally collected using an aseptic technique in a sterile environment, such as an OR, and are collected from sites where no growth of microorganisms is expected. Infections of sterile body fluids or tissues may be caused by aerobic, facultative anaerobic, and/or strict anaerobes.

The proximity of the collection site to adjacent sites, which may or may not have significant normal flora, helps to determine the number and kind of media used for isolate recovery. Prior to collection of the specimen, the site should be appropriately cleansed. When possible, collect the specimen prior to initiation of antibiotic therapy. Collect specimen while minimizing exposure to contamination by indigenous flora in adjacent areas.

While blood is considered a sterile body fluid, it is addressed in its own procedure. Refer to procedure MICRO.CULT.2.0 *Blood Culture*.

**OWNERS**

Supervisor, Regional Microbiology

**SPECIMEN**

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| Specimen type | Fluids include: pericardial, pleural, peritoneal, synovial, cerebrospinal (CSF), peritoneal dialysis fluid (PDF), etc.  Tissue or biopsy from any site  Aspirate  Fine needle aspirates (FNA) |
| Collection containers | Culturette swabs – least recommended choice  Sterile screw cap containers  Anaerobic transporters  Capped syringe with needle removed  Sterile blood collection tube without preservative (or with heparin, if preservative is required to prevent clotting of specimen)  Blood culture bottles |
| Volume | 1-5 ml desired for most fluids  50 ml minimum requested for PDF  As much tissue as possible |
| Stability and Storage Requirements | Prompt delivery to the laboratory is optimal. Store specimen at room temperature for up to 24 hours. Do not refrigerate. Small tissue samples or punch biopsies should have a few drops of sterile physiological saline added if the specimen is submitted in a sterile screw cap container. Dessication is deadly to most microorganisms. |
| Unacceptable specimens | Formalin preserved specimens  Dry swabs  Expired media or transporters  Swab for anaerobic culture submitted in an aerobic transporter. |

# MATERIALS

# Trypticase Soy agar with 5% Sheep Blood (BAP), store at 2-8°C until use

1. MacConkey agar (MAC), store at 2-8°C until use
2. Chocolate II agar (CHOC), store at 2-8°C until use
3. Columbia agar with Colistin and Nalidixic Acid (CNA), store at 2-8°C until use
4. CDC Anaerobic Blood agar (ABAP), store at 2-8°C until use
5. CDC Anaerobic Blood agar with PEA (APEA), store at 2-8°C until use
6. CDC Anaerobic Laked Blood agar with KV (AKV), store at 2-8°C until use
7. Thioglycolate with Vitamin K and Hemin (THIO), store at 2-8°C until use

# QUALTIY CONTROL

This procedure describes the process used to isolate, identify, and report bacteria from sterile fluid and tissue cultures. All media, reagents, biochemicals, and kits used in conjunction with this procedure shall be checked for proper reactivity using appropriate control organisms or manufacturer’s QC documents. Refer to specific test procedures or department QC plan for detailed quality control requirements.

**PROCEDURE**

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| If | Then |
| Specimen is a culturette swab | Use the swab to inoculate media |
| Specimen is a fluid with more than 1 ml received | Centrifuge the specimen at 3500 rpm for 15 minutes. Use the sediment to inoculate media. |
| Specimen is a tissue | Homogenize the tissue in a tissue grinder with 0.5 – 1.0 ml sterile saline. |

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| Step | Action |
| 1 | Use the culturette swab or a sterile transfer pipette to inoculate media according to the chart below |

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| If Specimen is | Then Inoculate |
| CSF  *(Gram stain and culture must be processed immediately upon receipt.)* | BAP and CHOC |
| Bone marrow or CSF from shunt | BAP, CHOC and THIO |
| Tissue, aspirate, joint (synovial) fluid, or fluid (other than CSF) from organs or internal source above the waist (ie. Heart, lung, brain, etc.) | BAP, CHOC AND ABAP |
| Tissue, aspirate, or fluid (other than CSF) from internal source at or below the waist or from skin or extremities (ie. Abdominal, peritoneal dialysate, hand, toe, etc.) | BAP, MAC, CHOC, CNA, ABAP, APEA, and AKV. |
| Tissue | Inoculate THIO in addition to plates. |
| Received inoculated in blood culture bottle(s) | Incubate bottle(s) on the BACTEC instrument for 5 days. Preliminary work up of any potential positive will occur at the blood bench. When a positive culture has been found, Gram stain results, plated media, and the original bottle(s) will be given to the routine bench for all additional work up. |

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| Step | Action |
| 2 | Streak the plates for isolation. |
| 3 | Incubate the aerobic media and THIO at 35-37°C in 5-10% CO2. |

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| If | Then |
| Anaerobic media is to incubated in the anaerobic chamber | Place plates in a metal rack. Label the rack with the day plates will be removed from the chamber (full 48 hours from inoculation). Immediately place the rack in the anaerobic chamber. |
| Anaerobic media is to be incubated in an anaerobic jar | Plate plates in rack within the jar. Place indicator and gas generating pack(s) in the jar and seal. Label the jar with the day plates will be removed from the incubator. Incubate the jar at 35°C in the walk-in incubator. Jars must not be opened for a full 48 hours from the time of inoculation. |

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| Step | Action |
| 4 | Prepare a Gram stain (refer to procedure U7.3). |

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| If | Then |
| Specimen is a clear or non-viscous fluid | Cytospin the specimen for the Gram stain. |
| Specimen is tissue, or a cloudy, bloody or viscous fluid. | Use the homogenate from grinding or the direct specimen to make the Gram stain. |

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| Step | Action |
| 5 | Read the prepared Gram stain and issue a report according to the Gram Stain procedure. |
| 6 | Examine the plated aerobic media daily for growth. |

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| If | Then |
| Specimen is sterile fluid (CSF, synovial, pericardial, etc)  Note: Does not include drainage cultures, POF or abdominal fluids. | Incubate the media 5 days for negative cultures. |
| Specimen is other than sterile fluid | Incubate the media for a full 48 hours. |

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| If | Then |
| There is no growth on the plates on day 1 | Reincubate the plates for an additional 24 hours and issue a preliminary report. |
| There is growth on the plates on day 1 | Quantitate\* and identify the microorganisms present and perform susceptibility testing (if indicated). Issue a preliminary report. |
| More than 2 species of intestinal flora observed in peritoneal fluid | Refer to procedure 1.3.28 Wound Culture procedure. Workup and report according to guidelines for mixed cultures. |

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| If | Then |
| There is no growth on the plates on day 2 (through day 5, if applicable) | Reincubate the media until incubation period is complete. Update the preliminary report to final upon completion. |
| There is growth on the plates on day 2 or through day 5. | Identify the microorganisms present and perform susceptibility testing. Report the quantity, identification, and susceptibility (if indicated) of the microorganisms recovered.  **NOTE: All enterococcal isolates recovered from sterile sites must be screened for β-lactamase production using the Nitrocefin disc procedure,** [**MICRO.AST.8.0**](http://maclsp64/soplib/Official%20SOPs/Beta%20Lactamase%20Test%20Using%20Chromogenic%20Cephalosporin-docx.pdf)**.** |

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| Step | Action |
| 7 | Examine the THIO daily for signs of growth (e.g. turbid, gassy, etc.). |

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| If | Then |
| THIO and plated media are no growth | No additional action required. Update the culture report as No growth. Discard THIO. |
| THIO shows signs of growth and plates have no growth. | Gram stain the broth and subculture to CHOC, BAP and ABAP. Additional media can be used based on Gram stain morphology seen. Save THIO for 1 week after culture is final. |
| THIO shows signs of growth and culture results show organisms reported in direct smear. | No additional action required.  Report the culture based upon growth on plated media.  Discard THIO. **Exception**: CSF shunt, heart valve, hardware, or internal organ source should have THIO gram stained to confirm same organism(s) are present in both broth and plated media, plate if needed, save THIO for 1 week after culture is final. |
| THIO shows signs of growth and culture results do not show organisms reported in direct smear. | Gram stain the broth and subculture to CHOC, BAP and ABAP. Additional media can be used based on Gram stain morphology seen. Save THIO for 1 week after culture is final. |
| THIO shows signs of growth and culture plates show≥3 organism morphologies | No additional action required. Report the culture based upon growth on plated media. Discard THIO. |

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| Step | Action |
| 8 | Examine ABAP, APEA, and AKV, if inoculated, after 48 hours of incubation. |
| 9 | Compare growth on anaerobic plates with growth on aerobic plates. |
| 10 | On growth of suspected anaerobes, perform Gram stain, aerotolerance check, and subculture to ABAP. Incubate aerotolerance check at 35°C in CO2 and subculture ABAP and original anaerobic plates at 35°C in anaerobic environment for 1-2 days. |
| 11 | Read and record results of aerotolerance check daily. |
| 12 | After subculture, compare growth on aerotolerance check and subculture ABAP to determine if the organism grows better anaerobically or aerobically. |

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| If | Then |
| 1-2 anaerobes are present on culture | Identify organisms using RapID ANA kits. |
| >2 anaerobes are present on culture | Report culture as Mixed anaerobic flora (MIXA/JIMIXA). |

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| 13 | Identify any aerobes that were not recovered on the aerobic plates. Record and perform work up under the anaerobic culture. When reporting, add a comment to the organism that indicates it is additional growth that was not recovered on the aerobic culture. *(Note: Report the organism under the aerobic culture if susceptibility testing is to be performed.)* |
| 14 | Save representative original plates, purity plates, and anaerobic subculture plates for 1 week |

### REPORTING RESULTS

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| Step | Action |
| 1 | Determine the quantity of each organism morphology present: |

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| If | Then | |
| Sunquest | QLS |
| 1-5 Colonies present | RARE | #RA |
| Non-confluent growth in the 1st quadrant only | FEW | #LG |
| Confluent growth in the 1st quadrant or growth in the 2nd quadrant | MOD | #MG |
| Growth in 3rd or 4th quadrant | MANY | #HG |

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| Step | Action |
| 2 | Issue report. |

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| If | Then | Preliminary | | Final | |
| Sunquest | Toplab | Sunquest | Toplab |
| There is no growth | Report no growth | / | #NGD | NG | #NG |
| There is growth | Report quantitation and organisms identified | Applicable code | Applicable code | Applicable code | Applicable code |

# PROCEDURE NOTES

1. Call positive CSF cultures to the submitting location.
2. When reporting CSF isolate susceptibilities, do not report the following: aminoglycosides (including gentamicin, tobramycin, and amikacin), first generation cephalosporins such as cefazolin and cephalothin, second generation cephalosporins such as cefamandole (with the exception of cefuroxime), clindamycin, quinolones, sulfas, erythromycin, tetracyclines, or cefoperazone. Refer to the CLSI Performance Standards for Antimicrobial Susceptibility Testing for further information.
3. Do not report the following antibiotics on systemic isolates; report them only on urine cultures. Nitrofurantoin, naladixic acid, norfloxacin, sulfisoxazole, and trimethoprim.
4. Refer to Procedure 9.16 Telephone Reporting Policy for required notifications and for a list of organism requiring notification or submission to the Indiana State Department of Health.
5. At the first indication of a possible *Neisseria meningitidis* or suspected agent of bioterrorism, perform all colony manipulations in a biological safety hood.
6. For cultures from lymph nodes, work in a biological safety cabinet, since pathogens can be found in these specimens that are hazardous, e.g. *Franciscella*, *Brucella*, and *Mycobacterium*.
7. Refer identification and susceptibility testing on morphologically identical isolates that are recovered from the same, or a similar specimen, type culture collected within 4 days.
8. Do not perform susceptibility testing on anaerobes, yeasts, *Streptococus* viridans group, corynebacteria, group D Non-Enterococcus species or *Neisseria* species.
9. Do not perform susceptibility testing on *Haemophilus* species. Only perform and report beta-lactamase testing.
10. Send anaerobe isolates with requests for susceptibility testing to Quest for their standard anaerobe panel susceptibility.
11. Grossly mixed specimens (containing ≥ 3 organism morphologies) may require consultation with the clinician to determine the appropriate course of action.

**LIMITATIONS**

1. Culture results are only as good as the specimen collected and submitted. Inadequate specimens may give erroneous or misleading results.
2. Patients receiving antibiotics or chemotherapy may have false negative culture results.
3. Pathogens not addressed in this procedure include fungi, mycobacteria, and viruses, among others. Additional procedures may be required to recover these and other pathogens.

# REFERENCES

1. Isenberg, Henry, ed. *Clinical Microbiology Procedures Handbook*, American Society for Microbiology, March 2007, 3.13.1.
2. Murray, Patrick R, ed. *Manual of Clinical Microbiology*, American Society for Microbiology, 1999.