PURPOSE: To determine the presence or absence of bacterial pathogens in sterile body fluid specimens.

SPECIMEN INFORMATION:

Commonly submitted types of body fluids submitted for culture:

Fluid	Synonym	Location
Pleural	• Empyema • Thoracentesis	Fluid within the membrane surrounding the lungs and the chest wall.
Peritoneal	• Abdominal • Ascites • Paracentesis	Fluid within the membrane lining the abdominal cavity.
Joint	• Synovial • Bursa fluid • Arthrocentesis fluid • Prosthetic joint fluid	Fluid at the union of two bones.
Pericardial		Fluid within the membrane lining of the cavity of the heart.
Cul-de-sac	• Culdocentesis	Fluid within the pouch between the wall of the rectum and the wall of the uterus.
Amniotic	• Amniocentesis	Fluid within the membrane of the fetus.
Other Fluids	Infection of normally sterile body fluids may result in severe morbidity and mortality. Any organism isolated must be considered significant (although specimen contamination may occur during collection). Specimens include: tympanocentesis fluid, intraocular fluid, hydrocele fluid, cyst fluid, etc.	

NOTE:

- **Refer to MIC34300 – Blood Products Culture for blood products.**
- **Refer peritoneal dialysis fluid specimens for culture to DynaLIFE.**
- **Refer prosthetic device specimens for culture to DynaLIFE.**
- **Refer tissue or biopsy specimens for culture to DynaLIFE.**

SAMPLE INFORMATION:

Special Precautions	Refer to Policy B-0160: Specimens Containing Suspected Risk Group 3 Pathogens for Primary Specimen Handling Flow Chart
Type	• Fluid should be collected in a sterile specimen container or tube and/or into blood culture vials • If fluid is received in blood culture vials, order as Blood Culture-Fluid and process as blood culture • If swab is received, add Specimen Quality comment **SWBFL** which states: **"Swab sample may be inadequate for recovery of organisms. Interpret results with caution"**
Source	Refer to chart on page 1.
Stability	Transport to the laboratory immediately
Storage Requirements	If a delay in processing is anticipated, hold specimens at room temperature, do NOT refrigerate
Criteria for rejection	1. Insufficient volume for tests requested: contact the physician to prioritize requests. 2. Leaking specimens should be processed, but alert the physician of the possibility of contamination. 3. Specimens received in the laboratory in a syringe with the needle still attached will be rejected. In addition, a RiskPro will be filed outlining the hazard. Refer to SCM40100 - Specimen Acceptance and Rejection Policy. 4. Improperly collected, labeled, transported or handled specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse. 5. If only blood culture vials are received, a gram stain cannot be performed.

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC), Colistin Nalidixic Acid agar (CNA), Brucella agar (BRU), Brucella Laked Blood agar with Kanamycin and Vancomycin (KV) and Thioglycollate broth (THIO)
- BACTEC Plus aerobic/F culture vials and BACTEC Plus lytic /10 anaerobic culture vials
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES

- Disposable inoculation needles
- Microscope slides
- Alcohol swabs
- Blue top conical tube
- Biosafety cabinet
- Anaerobic jar and pouch
- 35° ambient air and 35° CO_2 incubators
- Wooden sticks
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

Step	Action	
Processing specimens for body fluid culture		
1	>1 mL received	• Transfer specimen to sterile conical tube (located in TB lab). • Centrifuge at 3500 rpm for 10 minutes. • Transfer supernatant to labeled red top tube.
	<1 mL received	• Do not centrifuge.
2	In the biosafety cabinet, using a sterile pipette: • Aspirate the sediment from the bottom of the conical tube. • Place 1 - 2 drops each onto BA, CHO, MAC, BRU and KV. Add 2 to 5 drops to THIO. • Streak for isolated growth using a disposable inoculation needle. Streak out to cover the whole plate. • Prepare smear by placing 1 or 2 drops of fluid on a cytology ringed microscope slide. Clean slide with alcohol swab prior to inoculation.	
3	Label THIO with Day 2 date and Day 5 date. Place in THIO rack in O_2 incubator in "Day 2" row.	
4	Place conical tube, supernatant tube and MAC in the O_2 incubator. Place BA and CHO plates in the CO_2 incubator.	
5	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with date of 48 hours read. Anaerobes should not be exposed to air for 42-48 hours after inoculation.	
6	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.	
7	Interpret fluid stains immediately. During the regular Microbiology lab hours of 08:00 to 20:00, turnaround time for these gram stains is <1 hour. Outside the regular Microbiology lab hours, Microbiology Technologist may be called in if ordering physician determines the stain must be read immediately.	
8	Immediately phone results of any positive stain results for microorganisms to ordering location and document the conversation within the LIS.	
9	Examine aerobic plates after 24 hour incubation. Record observations in the LIS.	
10	Re-incubate CO_2 plates for an additional 48 hours. Re-incubate O_2 plate for an additional 24 hours.	

11	At 48 hours, examine plates and record observations in the LIS.
12	At 72 hours, examine plates and record observations in the LIS.
13	Examine anaerobic plates after 48 hours incubation and record observations in the LIS. Re-incubate BRU anaerobically for an additional 72 hours. If specimen is from the neck or above, re-incubate BRU for an additional 8 days. After 5 or 8 days, as applicable, examine plate and record observations in the LIS.
14	Examine THIO on day 2 and day 5 for growth and record observations in the LIS. If growth is suspected, perform gram stain. If gram stain resembles growth on aerobic plates, subculture of broth is not indicated. If growth does not resemble growth on aerobic plates, subculture broth to CHO, incubated in CO_2 and BRU, incubated anaerobically.

Probable Pathogens		
• *Actinomyces* spp. • *Arcanobacterium* • *Aeromonas* • *Bacillus anthracis**+ • *Bacteriodes fragalis* • β-hemolytic streptococci • *Brucella* spp.*+ • *Campylobacter* • *Candida* spp. • *Capnocytophaga* spp. • *Eikenella corrodens*	• Enterobacteriacea • *Erysipelothrix* • *Francisella**+ • Molds • *Haemophilus influenzae* • *Helicobacter* • *Kingella kingae* • *Listeria* spp. • *Moraxella catarrhalis* • *Neisseria gonorrhoeae*	• *Neisseria meningitides**+ • *Nocardia* spp. • *Pasteurella multocida* • *Pseudomonas aeruginosa* • *Staphylococcus aureus* • *Staphylococcus intermedius* • *Staphylococcus lugdunensis* • *Streptococus anginosis* grp. • *Streptococcus pneumoniae* • *Vibrio* spp.

Potential Pathogens	
• *Aggregatibacter* spp. • Anaerobes other than *Bacteriodes fragilis* • *Bacillus* spp. • *Corynebacterium* spp. • *Enterococcus* spp. • *Haemophilus* spp. other than *H.influenzae* • *Lactobacillus* spp.	• *Micrococcus* spp. • *Moraxella* spp. other than *Moraxella catarrhalis* • Gram-negative, non-fermenters other than *Pseudomonas aeruginosa* • Coagulase-negative *Staphylococcus* • *Staphylococcus* spp. other than those listed as “pathogens”

*** Risk group 3 organism. If suspected, refer to Policy B-0160: “Specimens Containing Suspected Risk Group 3 Pathogens” for Primary Specimen Handling Flow Chart.**

+ All work should be performed in the BSC.

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of body fluid specimens	
1	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	Observe aerobic plates at 24 hours, 48 hours and 72 hours for growth. Count the number of types of organisms growing.
3	**>=3 organism growing on any media:** • Consult DynaLIFE microbiologist.
4	**1 – 3 organisms growing on >1 media:** • <u>If organism(s) is a pathogen</u>: ➢ Perform identification and susceptibility testing. • <u>If organism(s) is a potential pathogen</u>: ➢ Perform identification and susceptibility testing if ANY of the following are true: o Organism(s) is intracellular in direct smear. o Organism(s) is pure or predominant in direct smear. o Organism pure on culture o Multiple or previous cultures are positive for the same organism(s). ➢ If NONE of the above is true, perform identification and list organism(s).
5	**1 – 3 organisms growing on 1 medium only, THIO broth:** • <u>If organism(s) is aerobic</u>: ➢ Perform identification and susceptibility testing if ANY of the following are true: o Organism(s) is a pathogen o Organism(s) is intracellular in direct smear o Organism(s) is pure or predominant in direct smear o Multiple or previous cultures are positive for the same organism(s) ➢ If NONE of the above is true, perform identification and list organism(s).

5	**1 – 3 organisms growing on 1 medium only, THIO broth con't:** • <u>If organism is pure growth and anaerobic</u>: ➢ If anaerobic organism is pure, refer to DynaLIFE for identification and susceptibility testing if ANY of the following are true: o Organism is a pathogen o Organism is intracellular in direct smear o Organism is pure or predominant in direct smear o Multiple or previous cultures are positive for the same organism ➢ If NONE of the above are true, list identification based on gram stain results (i.e. anaerobic gram-negative bacilli) • <u>If there are ≥ 2 anaerobic organisms</u>: ➢ Consult DynaLIFE microbiologist if ANY of the following are true: o Organism(s) is a pathogen o Organism(s) is intracellular in direct smear o Organism(s) is pure or predominant in direct smear o Multiple of previous cultures are positive for the same organism ➢ If NONE of the above are true, list identification based on gram stain results (i.e. anaerobic gram-negative bacilli).
6	**1 – 3 organisms growing on 1 solid medium only (THIO clear):** • <u>If organism(s) is present in the direct smear</u>: ➢ Perform identification and list organism(s). ➢ Consult DynaLIFE microbiologist for susceptibility testing required. • <u>If organism(s) is not present in the direct smear (possible lab contaminant)</u>: ➢ Report culture as "No growth" if ALL the following are true: o Organism(s) is not a pathogen or potential pathogen o Organism(s) colony distribution if suggestive of contaminant o No current or previous cultures are positive for the same organism(s) ➢ Consult DynaLIFE microbiologist if ANY of the following are true: o Organism(s) is a pathogen or potential pathogen o Colonies are on the streak line or inoculum o Multiple or previous cultures are positive for the same organism(s)

REPORTING RESULTS:

IF	REPORT
No growth or organism determined to be laboratory contaminant after 1 day	**PRELIM:** • Report: **"No Growth after 1 Day. Further report to follow"**
No growth or organism determined to be laboratory contaminant on aerobic media after 3 days	**INTERIM:** • Report: **"No growth aerobically after 3 days"** • Report: **"@Anaerobic culture to follow"**
No growth or organism determined to be laboratory contaminant on anaerobic media after 5 days	**FINAL:** • Report: **"No anaerobes isolated after 5 days"**
No growth or organism determined to be laboratory contaminant on anaerobic media after 5 days and specimen source is from the neck or above	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AC10** to state: **"The anaerobic culture will be incubated for an additional 5 days for the isolation of Actinomyces spp. A further report will follow only if positive".**
Growth of non-significant organism(s) in THIO broth only	• Report organism(s) identification under the isolates tab. • List quantitation as: **"Isolated from Enrichment Broth"** • Add isolate comment **&THIO** to state: **"Isolated from enrichment broth only. Clinical correlation required. If clinical diagnosis is infection, please contact the microbiology laboratory at 669-4162 for susceptibility testing."** • Freeze organism(s) and log into stored isolate log.
Growth of significant organism(s) in THIO broth only	• Report organism(s) identification under the isolates tab. • List quantitation as: **"Isolated from Enrichment Broth"** • Report susceptibility results as per ASTM. • Freeze isolate(s) and log into stored isolates log.

Growth of pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM.
Pure growth of anaerobic organism	• Report organism based on gram stain results under the isolates tab. • List quantitation. • Add isolate comment **&REF4** to state: **"This organism has been referred for further identification and susceptibility testing."** • Refer organism to DynaLIFE for identification and susceptibility testing as per MIC10510 – Referral of Category B Specimens to DynaLIFE. • Use anaerobic transport media. • Freeze isolate and log into stored isolates binder.
H. influenzae or N.meningitidis isolated	• Any sterile body fluid specimen positive for *H.influenzae* or *N.meningitidis* must be sent immediately to Provincial Lab for typing as soon as identification is confirmed. Assure there is a purity plate made that can be used for this purpose and can be sent out the day the identification is confirmed. Refer to MIC10510.
S.pyogenes, S.agalactiae, S.pneumoniae, H. influenzae or N.meningitidis isolated	• Any *S.pyogenes*, *S.agalactiae, S.pneumoniae, H.influenzae* or *N.meningitidis* isolated from CSF specimens must be sent to NML for International Circumpolar Surveillance (ICS) program. Refer to MIC10520.

NOTE:

- **Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1.**
- **Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control.**
- **Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.**

CULTURE NOTES:

- Report the probable identification of the organism as soon as possible.
- Generally, a positive culture indicates infection with the organism.
- WBC are usually present with infections of body fluids.

LIMITATIONS:

1. False-positive cultures can result from contamination of the specimen with skin flora.
2. False-negative results can be caused by low numbers of organisms, prior antimicrobial treatment or the fastidious nature of the infective organism.
3. Body fluid swabs are not ideal specimens and should be noted in the specimen quality section of order entry.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven