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| Body Fluid Culture |
| **Purpose** | This procedure provides instruction for Body Fluid Culture for the Microbiology Laboratory. |
| **Principal and Clinical Significance** | Infection of normally sterile body fluids often results in severe morbidity and mortality. Most microorganisms infecting these sites are not difficult to culture, but occur in low numbers. Therefore, even one colony of a potential pathogen may be significant. All isolates from these sites must be reported. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | BF |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
 | * Glass slide
* Sterile disposable pipette
* Sterile container
* Anaerobic gas pack
* Anaerobic jar
 | * Ambient air incubator
* Centrifuge
* CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
* Vortex mixer
* BactecTM blood culture instrument
 | Refer to the Sunquest specimen label for media information. The specimen site determines appropriate media.* Chocolate agar (CHOC)
* Sheep blood agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)
* Thioglycollate (THIO)
* BactecTM Peds Plus/F aerobic medium, pink cap (BPNK)
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| **Specimen** | 1. Acceptable specimens: Aseptically aspirated body fluid (excludes cerebrospinal fluid, blood, and urine) from a normally sterile site.
2. Indicate if the specimen was received on a swab (ABDF-SWAB). Swabs are the least desirable specimens since the quantity of sample may not be sufficient to recover small numbers of organisms. Report the following disclaimer “NEGATIVE RESULTS ARE UNRELIABLE FOR SPECIMENS OBTAINED ON SWABS” (Sunquest code: **NSWAB.)**
3. Invasively collected specimens in leaky containers must be processed. Indicate in the report that there may be a possibility of contamination due to a leaking container.
4. Special instructions
* Aliquot the appropriate amount of specimen for other requests such as AFB Culture or Fungal culture. If there is a small amount of specimen, call the physician to prioritize the tests.
* Process immediately

Refer to the Lab Test Directory for further information [Body Fluid Culture and Gram Stain](https://www.childrensmn.org/References/Lab/microbioviral/body-fluid-culture-and-gram-stain.pdf) |
| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
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| **Procedure** | **A. Inoculation**Warm all media before inoculation.Label all plates, tubes and slides properly with the patients name, accession number and date.1. Inoculate the media in the order of the least selective first to prevent carryover of inhibitory substances to another medium. Refer to the Sunquest specimen label for the order of inoculation.
2. Joint fluid requires inoculation of the BPNK first to enhance the recovery of *Kingella kingae.*

 Example: Joint fluid (JF): BPNK, CHOC, SB, GMSTAlways inoculate the culture media first before preparing the slide when using the same pipette.Specimen processingFollow the **fluid** and/or **volume** specific instructions listed below:1. **Received in a Blood culture bottle only (inoculated on floor or in O.R.):**
2. Perform a blind subculture from the bottle to a CHOC before Bactec insertion.
3. **Less than 0.3 ml of fluid:**
4. Do not centrifuge.
5. If the specimen is only 1 or 2 drops, rinse the syringe with a small amount of TSB.
6. Place one drop on CHOC.
7. Place ½ drop on a slide for Gram stain.
8. Place the remaining fluid into a BPNK or THIO (refer to Sunquest label).
9. **Greater than 0.3 but less than 1 ml of fluid:**
10. Do not centrifuge.
11. Place one drop on CHOC and SB.
12. Place one drop on glass slide for Gram stain. If the specimen is viscous, bloody or has pus, spread on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.
13. Put remaining fluid into or BACTEC™ bottles or THIO (refer to Sunquest label).
14. **Greater than 3 ml of fluid:**
15. Inoculate BACTEC™ bottle(s), 1 mL minimum, (if indicated on Sunquest label).
16. Place one drop of specimen on CHOC and SB.
17. Place one drop on glass slide for Gram stain. If the specimen is viscous, bloody or has pus, spread on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick. If the fluid is clear, use the cytocentrifuge to concentrate the specimen for the smear.
18. Put remaining fluid into THIO (if indicated on Sunquest label).
19. **Joint fluid:**
20. Inoculate the BPNK bottle first with 0.25-1.0 ml of fluid. If the volume is only a few drops, rinse syringe with a small amount of broth from the BPNK to remove entire specimen from syringe. Omit Gram stain and plates on low volume specimens. Inoculate the BPNK only.
21. Place one drop on CHOC and SB.
22. Place one drop on glass slide for Gram stain. If the specimen is viscous, bloody or has pus, spread on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.
23. **PD fluid ≥10 ml**
24. Inoculate 3 ml of fluid into aerobic blood culture medium.
25. Place one drop on CHOC and SB.
26. Place one drop on glass slide for Gram stain.
27. **Clotted specimen**
28. Put clotted specimen into a sterile tissue grinder or stomacher bag.
29. Add 0.5 ml of sterile saline and gently homogenize to disperse clot and release bacteria.
30. Streak plates semi-quantitatively for primary isolation.
31. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
32. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
33. Flame the loop, turn the plate a quarter turn and pass the loop through the edge of the first quadrant approximately 4 times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant 3-4 times.
34. Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 4-5 times.

~AUT00291. **Incubation**
2. Incubate CHOC, SB, and CNA in 4-10% CO2 at 35ºC.
3. Incubate MAC and THIO in ambient air incubator at 35ºC.
4. Place BACTEC™ bottle in the BACTEC™ blood culture instrument by using barcode entry. Enter patient demographics using the manually.
5. **Gram stain examination**

Perform Gram stain and interpret.1. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
2. Notify provider of positive results. See Result Reporting for more information.
3. Blot excess oil from slide. Hold slide for one week.
4. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.
5. **Culture examination:** Read plates daily for 3 days for invasively collected specimens and 2 days for drainage specimens.
6. Day 1
7. Examine aerobic plates and broth.
8. Plated media
9. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, etc.
10. Correlate colony types with the direct Gram stain.
11. Use the initial Gram stain to help determine the extent of work-up required on the culture. The presence of many WBCs indicates an infectious process.
12. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
13. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
14. Subculture organisms that are not well isolated to appropriate media for further work-up.
15. Re-incubate primary plates and subcultures for an additional day.
16. Report preliminary results. Call results that are critical values.
17. THIO broth
18. Visually inspect THIO.
19. If growth is observed, perform gram stain on THIO.
20. Correlate the culture result with the Gram stain of the THIO. Do not subculture the THIO if the gram stain correlates with the growth on the plates. However, if the culture grew out CNS and Gram-positive cocci are seen in the broth, subculture the broth to rule out *S. aureus.* Discard after 2 days.
21. If there appears to be additional organisms in the THIO that are not on the plates, determine if Anaerobic Culture has been ordered. If Anaerobic Culture has been ordered, subculture to appropriate aerobic media. Identify appropriate organisms. If organism in THIO appears to be an anaerobe, hold THIO for 5 days. After 4-5 days, confirm isolation of organism in Anaerobic Culture before finalizing culture. If Anaerobic Culture has not been ordered, subculture to appropriate aerobic and anaerobic media. Identify appropriate organisms. Add bill code ANAID.
22. BACTEC™ bottles
23. Perform subculture to CHOC and SB and gram stain all positive bottles.
24. Select additional media (CNA/MAC/SAB/CCAN) and inoculate media based on the gram stain result. CNA/MAC if gram neg rods seen. SAB/CCAN if yeast seen.
25. If subculture plates from the Bactec™ bottles are the same as original plates, perform minimal work-up.
26. Set up additional tests as needed.
27. If bottles are negative, continue to incubate BACTEC™ bottles for 5 days.
28. Day 2
29. Examine primary plates from the previous day for additional microorganisms.
30. Read and record identification tests and susceptibilities from the previous day.
31. Set up additional tests as needed.
32. Visually inspect THIO. If growth is observed, perform gram stain on THIO. Refer to section ‘c’ above for further instructions.
33. Ensure THIO with growth was gram stained for 2 consecutive days.
34. Consult micro lead tech when the identification or extent of the identification is not clear. Delays in identification can affect patient care.
35. Send updated report.
36. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate. Freeze isolate for future reference.
37. **Send invasive pathogens cultured from sterile fluids to MDH for the EIP program.**
38. Additional Days
39. Complete identification and susceptibility testing procedures until all significant isolates are finished.
40. Send updated report and finalize.
41. If there is no growth on the plates, they can be tossed at 3 days. Culture is held open while THIO continues to Incubate.
42. Hold negative THIO for 5 days. If no growth in THIO, final report as “No Growth, 5 Days”.
43. Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 14 days in the red save boxes in case a physician calls for further studies.
44. Save a representative primary plate for anaerobes in an anaerobic jar or bag, whether a complete work-up was performed or not, at room temperature for 14 days in case a physician calls for further studies.
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| **Limitations** | Negative results are unreliable for specimens collected on swabs. |
| **Method Performance Specifications** | 1. Emerging infections program (EIP): Send invasive pathogens to MDH cultured from sterile sites. Sterile sites include pleural fluid (chest and thoracentesis fluid), peritoneal fluid, pericardial fluid, and joint fluid.

Invasive Pathogens: *Neisseria meningitidis* *Haemophilus influenzae* *Beta streptococcus group A* *Beta streptococcus group B* *Streptococcus pneumoniae* *Kingella kingae*1. For culture positive with one or two organisms, perform definitive identification and antimicrobial susceptibility testing.
2. For cultures with greater than three organisms, perform definitive identification and antimicrobial susceptibility testing if one or two organisms are predominant.
3. Perform limited identification and no susceptibility testing on the following:
4. Probable skin contaminants: One or two colonies of CNS on one plate and no growth in the broth.
5. For peritoneal specimens that contain mixed gastrointestinal flora and no predominant organism, report as “MIXED FLORA, no further identification” (**MF**) or “MIXED ANAEROBIC FLORA, No further identification” (**MIXA**). Hold the plates for further testing if requested. EXCEPTION: If yeasts, *S. aureus, S. pyogenes,* or *P. aeruginosa* are isolated, they should be listed separately and not included in the “mixed flora”.
6. If unable to determine clinical relevance, consult with physician or lead tech.
7. Do not grind clots if a fungal culture is ordered. Tease the clots apart. Grinding will destroy fungal cells.
8. If *Brucella* is suspected, extend incubation time of Bactec™ bottles to 10 days.
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| **Result Reporting** | 1. Critical Value: Report positive Gram stains and cultures from normally sterile sites by telephone to the physician or patient’s nurse. Document in the computer the person called and the date/time of the call.
2. Culture results: Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box. Report results semi-quantitatively, i.e., 1+, 2+, 3+ or 4+.

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| Quantity | 1st quadrant# colonies | 2nd quadrant# colonies | 3rd quadrant# colonies |
| 1+ | <10 |  |  |
| 2+ | >10 | <5 |  |
| 3+ | >10 | >5 | <5 |
| 4+ | >10 | >5 | >5 |

1. No growth cultures: Update culture status in the Observation result box (*Culture Entry* tab), by using the “No Growth” update key (‘). Report as “No growth “*x*” days". Final (/) culture at 5 days.
2. Positive cultures:

Observations: 1. 4+ STAPHYLOCOCCUS AUREUS Further identification to followWorkups: Wkup # 1 Workup Components Med : SB GMS : STPH Desc : BH SC : SB Id : SAUR SLC : POS MSID : 1 VMIC : 1 1. If growth is only in the THIO or BACTEC™ bottles, report as:

Observations: 1. GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY Further identification to follow (**GNR-BO-FID**)Workups: Wkup # 10 Workup Components Med : THIO SC : SB MAC Desc : CLDY GMS : GMNR ID : GNR1. Gram stains: Report Gram stain results by selecting the *Direct Exam* tab. Follow Gram stain procedure for interpretation and resulting.

Observations: 1. 2+ GRAM POSITIVE COCCI 2. 4+ WBC'S1. Review **Culture Summary** for accuracy before filing report.
2. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Document date and time called in computer.

3. 3+ METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\*4. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.5. \*\*Called to Linda S., RN L8 @ 1300 7/7/031. If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code SRPT (supplementary report) must be used in SREQ or *Culture Observations* as follows:
* Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.
* Requests for additional testing: In the *Misc. Updates* tab, enter SRPT in SREQ followed by the request.
* Re-final the culture when identifications and/or testing are complete.

If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to the procedure [*Labeling Errors/Specimen Mix-ups and Correcting Patient Data*](file://\\kidsnet.childrenshc.org\chcdfs\dept\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MC%20100%20%20%20%20Quality,Spec.%20mgmt.,Labeling,Proc.,Sendout%20Results,Billing,%20PT%20testing,Addl%20Projects\MC%20102%20%20%20Labeling%20Errors,%20Specimen%20mixups,%20Corrected%20reports.doc) |
| **References** | 1. Pezzlo, M., Section 2. Aerobic bacteriology, 2.4, pg. 63 – 66. *In* H.D. Isenberg (Ed) *Essential Procedures for Clinical Microbiology.* 1998, American Society for Microbiology, Washington, D.C.
2. Leber, Amy, Section 1 & 3, Aerobic bacteriology, 1.8 & 3.5.6, *Clinical Microbiology Procedures Handbook*, 2016, American Society for Microbiology, Washington, D.C.
3. Yagupsky, P. 1999. Use of blood culture system for isolation of *Kingella kingae* from synovial fluid. J. Clin. *Microbiol.* 37:3785. (letter)
4. Yagupsky, P., R. Dagen, C. Howard, M. Einhorn, I. Kassis, and A. Simu, 1992. High prevalence of *Kingella kingae* in joint fluid from children with septic arthritis revealed by Bactec blood culture system, *J. Clin. Microbiol.* 30:1278-1281.
5. Lejbkowicz, F., L. Cohn, N. Hashman, and I. Kassis. 1999. Recovery of *Kingella kingae* from blood and synovial fluid of two pediatric patients by using BacT/Alert system. *J. Clin. Microbiol.* 37:878(letter).
6. Hughes, J., E. Vetter, R. Patel, C. Schleck, S. Harmsen, L. Turgeant, F. Cockerill, 2001. Culture with Bactec Peds Plus/F bottle compared with conventional methods of detection of bacteria in synovial fluid. *J. Clin. Microbiol.* 39:4468-4471*.*
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| **Appendices** | BATTERY: BFSPEC MEDIA0 CENT, CHOC, SB, THIO, GMSTABDF CENT, CHOC, SB, CNA, MAC, GMSTANAS BPNK, CHOC, SB, GMSTANK BPNK, CHOC, SB, GMSTDIAL BPNK, CENT, CHOC, SB, GMSTELB BPNK, CHOC, SB, GMSTGASP CENT, CHOC, SB, CNA, MAC, GMSTHIP BPNK, CHOC, SB, GMSTJF BPNK, CHOC, SB, GMSTKNEE BPNK, CHOC, SB,, GMSTNGF CENT, CHOC, SB, CNA, MAC, GMSTPD BPNK, CENT, CHOC, SB, GMSTPF CENT, CHOC, SB, CNA, , MAC, GMSTPLF CENT, CHOC, SB, BPNK, GMSTSF BPNK, CHOC, SB, GMST |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Pat Ackerman | 1973 | Initial Version |
| 1.1 | Pat Ackerman | 01/1992 |  |
| 1.2 | Pat Ackerman | 07/06/2003 |  |
|  | 1.3 | Pat Ackerman | 08/08/2004 |  |  |  |
| 1.4 | Pat Ackerman | 01/22/2006 |  |
| 1.5 | Pat Ackerman | 12/27/2006 | Added EIP information in Day 2 reporting and Procedure notes #1. |
|  | 1.6 | Pat Ackerman | 06/02/2007 | Updated Sunquest 6.2 reporting information. Specimen processing: 1. d. If the fluid is clear, use the cytocentrifuge to concentrate the specimen for the smear. |
|  | 1.7 | Becky Carlson | 10/12/2009 | Revised BactecTM bottle protocol to 5 days from 7 days. |
|  | 1.8 | Jessica Craig | 05/19/2010 | Updated into online format. |
|  | 2 | Becky Carlson  | 4/14/2015 | Re-numbered from MC 406 for CMS |
|  | 3 | Susan DeMeyere | 9/7/2017 | Changed reporting to keep culture open while THIO is incubating.  |
|  | 4 | Susan DeMeyere | 11/24/2017 | Remove centrifuging, decanting and resuspending the sediment.  |
|  | 5 | Susan DeMeyere | 10/31/2018 | Removed culturing for anaerobes on initial set up. Added instructions for THIO processing. |
|  | 6 | Susan DeMeyere | 10/25/2020 | Removed use of Scant when growth only from THIO. |