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| Wound Culture (Surgical or Deep) |
| **Purpose** | This procedure provides instruction for WOUND CULTURE (surgical or deep) for the Microbiology laboratory. |
| **Policy Statements** | This procedure applies to Microbiologists/Virologists who perform culture set-up and plate reading. |
| Principle and Clinical Significance | Wound infections can occur as complications of surgery, trauma and bites or diseases that interrupt mucosal or skin surfaces. The patient’s own flora or organisms in the hospital environment can be sources of surgical wound infections. Human and dog bite infections are often polymicrobic and grow the normal oral flora from the source. *S. aureus,* group Astreptococci, anaerobic cocci, *Clostridium* sp., members of the *Enterobacteriaceae, Bacteroides* sp., and *Fusobacterium* sp. are commonly associated with wound infections. Infections can range from simple postoperative infections to complicated, severe, and rapidly progressive infections such as necrotizing fasciitis.  Interpretation of the culture results should be based on the Gram stain criteria and laboratory testing. |
| **Test Code** | WDC |
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|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
| **Materials** | * Gram Stain reagents
 | * Glass slide (GMST)
* Anaerobic Gas pack
* Sterile dispo pipette
* Sterile tube
 | * Ambient air incubator
* Anaerobic jar
* CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
* Vortex mixer
 | Refer to the Sunquest specimen label for media information. Appropriate media is determined by the specimen site.* Chocolate agar (CHOC)
* Sheep Blood agar (SB)
* Anaerobic sheep blood agar (ASB2)
* Anaerobic Kanamycin-Vancomycin agar (AKV)
* CNA agar (CNA)
* MacConkey agar (MAC)
* Thioglycollate (THIO)
* Saline, Normal 1 mL (SLNE)
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| **Sample** |  |
|  |  | **Related document** |
|  | 1. Acceptable specimens
* Aspirated material or swab
* Aspirated material is superior to a swab specimen. If a swab must be used, collect two, one for culture and one for Gram stain.
1. SDES codes/Specimen type
* The code Sunquest code WND cannot be used as a SDES code. It is considered a source code and will error in Cerner PowerChart.
* State specific site of specimen. If the SDES CODE is unknown, do a keyword look-up at the SDES prompt by clicking on the *Result code lookup* button. Type in text and do a search by description. Highlight code and click on the **Add to list** button. Select the highlighted code to enter in SDES.

Text: ABDSearch option ○ Code ◙ DescriptionCode DescriptionABD ABDOMENABDF ABDOMINAL FLUIDAdd to List* Free text may be added to the specimen description code by clicking **Free Text**. Type free text in the box and click **OK**. This will automatically append the free text on to the SDES code. Click **Select** to save.
1. Specimen Collection and Transport
* Refer to [*Lab Test Directory - Deep wound/surgical culture*](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033676.asp)
1. Specimen assessment
* Refer to the policy MCVI 2.1 Specimen Rejection Criteria
 | [Lab Test Directory - Deep wound/surgical culture](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033676.asp) |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. Biohazard Containment
2. Safety in the Microbiology/Virology Laboratory
* Biohazardous Spills
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| **Procedure** | InoculationWarm 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. Always inoculate the culture media first before preparing the slide when using the same pipette.

Specimen processingAspirates and exudatesIf specimen is received in a syringe, transfer the entire amount into a sterile tube and mix well (vortex)If the specimen is received in a syringe and the volume is small, rinse syringe with a small amount of THIO or SLNE to remove the specimen from the syringe. Mix well.Place 1-2 drops directly on each plate, into the THIO and onto a slide.Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.Specimens received on swabs1. If anaerobic plates are to be set up, inoculate first by rolling swab over the upper quadrant of each plate.
2. Emulsify swab in 1.0 ml of sterile saline and vortex.
3. Squeeze the swab against the side of the tube to express remaining fluid and then discard.
4. Place 1-2 drops of the suspension directly on each plate, into a THIO. Place one drop of specimen on a slide for Gram stain.
5. Streak plates semiquantitatively for primary isolation.
6. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
7. Pass the loop back and forth through the inoculum in the first quadrant several times.
8. 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.
9. 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.

1. Incubation
2. Incubate CHOC, SB, and CNA in 4-10% CO2 at 35ºC
3. Place ASB2 and AKV in anaerobic holding chamber to be closed in an AnaeroPack™ System or GasPak anaerobic bag. Close for 2 days.
4. Place MAC, anaerobic bag or jar and THIO in ambient air incubator at 35ºC. Hold THIO for 5 days.
5. Gram stain examination

Perform Gram stain and interpret.1. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
2. Blot excess oil from slide. Hold slide for one week.
3. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.
4. Culture examination
5. Day 1:
6. Examine aerobic plates and THIO.
7. Plated media
8. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, etc.
9. Correlate colony types with the direct Gram stain.
10. 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. Squamous epithelial cells represent contamination
11. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
12. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
13. Subculture organisms that are not well isolated to appropriate media for further work-up.
14. Reincubate primary plates and subcultures for an additional day.
15. Report preliminary results.
16. THIO broth
17. Visually inspect THIO.
18. If positive, smear THIO for 2 consecutive days.
19. Correlate the culture result with the Gram stain of the THIO. Do not subculture the THIO if the smear correlates with the growth on the plates. Discard after 2 days.
20. If there appears to be additional organisms in the THIO that are not on the plates, subculture to appropriate media.
21. Day 2
22. Examine primary plates from the previous day for additional microorganisms.
23. Examine anaerobic plates, compare to aerobic plates, and do appropriate subcultures and identifications.
24. Read and record identification tests and susceptibilities from the previous day.
25. Set up additional tests as needed.
26. Send updated or final report.
27. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Freeze for future reference.
28. If there is no growth on the plates, discard after 2 days. Culture is help open while THIO is incubating.
29. Hold the THIO for 5 days. If no growth in THIO, final the report as “No Growth, 5 days”.
30. Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.
31. Additional Days
32. Complete identification and susceptibility testing procedures until all significant isolates are finished.
33. Send updated report and finalize.
34. **Send invasive pathogens cultured from sterile sites to MDH for the EIP program.** Refer to MCVI 4.0 and MCVI 4.1 MDH EIP for further information.
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| **Limitations** | Specimens are routinely screened for rapid growing anaerobes (e.g., *Bacteroides fragilis* group, *Clostridium perfringens*, *Fusobacterium*, and anaerobic gram-positive cocci). Slow-growing *Mycobacterium* sp. or *Nocardia* sp. that may cause abscesses will **not** be recovered in routine bacterial cultures even if present, since extended incubation periods or special media are necessary for their isolation. Cultures for these organisms should be specifically requested. |
| **Method Performance Specifications** | 1. Perform definitive identification and susceptibility testing on the following:
	* 1. Organisms isolated in **pure culture** and observed in the specimen Gram stain.
		2. Organisms isolated from **patients with catheter-related infections**
		3. Any quantity of a **probable pathogen**, i.e., *S. aureus, Ps. aeruginosa,* etc.
		4. Predominate to moderate numbers of **potential pathogens** with unpredictable susceptibility patterns such as the *Enterobacteriaceae.*
		5. If only one or two species are present or predominant and WBCs are seen on the smear, identify and perform AST.
		6. If >2 species with no predominant strain, perform ID only, no AST.
		7. Perform AST on multiple GNRs only on special request.
	1. Because of their known **virulence factors**, always report the following, even if not predominant:
2. Beta-hemolytic streptococci
3. Clostridium perfringens
4. Identify and report *Bacteroides* sp., *Fusobacterium* sp.
5. If three or more anaerobe species are present, report “Mixed anaerobic flora, no further identification.”

 * 1. If **yeast** is predominant or numerous or from a sterile site, identify to the species level.
	2. Identify organisms that are **always considered pathogenic**, i.e., *Brucella, Haemophilus, Pasteurella,* and *Francisella.* These organisms do not grow on MAC. *Francisella* and *Brucella* can be found in lymph node biopsies and are extremely infectious. *Francisella* is a tiny coccobacillus that grows slowly and is catalase positive and oxidase negative. *Brucella* is also a tiny slow growingcoccobacillus that is catalase, oxidase positive and urease positive. Refer to [*LRN Level A Bioterrorism Laboratory Protocols*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20210%20%20%20Updated%20Guidelines%20for%20LRN-%20Bioterrorism%20Procedure) for identification procedures.
	3. ***Viridans group streptococci* or *Enterococci***

a. Identify at least to the genus level from surgically, invasive specimens.b. The anginosis group (“S. milleri”) is often associated with brain and liver abscesses. Identify to the species level.*c. S. bovis* is associated with gastrointestinal malignancies.* 1. Perform limited identification and no susceptibility testing on the following:
		1. **Probable skin contaminants**, which include:

Coagulase-negative staphylococci diphtheroidsViridans streptococci *Bacillus* sp. with many epithelial cells/no PMNS* + 1. Isolates from sources such as **decubitis ulcer**, **perianal abscess** or **fistula** and **intestinal drainage.**
		2. **Mixed cultures, more than 3 organisms generally represent contamination or colonization**. If the culture grows more than 3 organisms, none that is predominant, report as “MIXED FLORA, no further identification” (**MF**) or “MIXED ANAEROBIC FLORA, No further identification” (**MIXA**).
		3. Hold the plates for further testing if requested.
		4. EXCEPTION: **If one organism is clearly predominant**, perform susceptibility testing.
	1. **Consult with physician on questionable cultures** regarding work-ups.
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| **Result Reporting** | 1. **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 semiquantitatively, 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 TUC : VMIC : 1 FOXS : 25-SS DTEST : POSIf growth is only in the THIO, report as:Observations: 1. SCANT GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY Further identification to follow (**SCAN-GNR-BO-FID**)Workups: Wkup # 10 Workup Components Med : THIO SC : SB MAC (Add Wkld: 2) Desc : CLDY GMS : GMNR ID : GNR1. **Gram stains**: Report Gram stain results by selecting the *Direct Exam* tab. Follow MC 3.0 Gram stain procedure for interpretation and resulting.

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

1. 3+ METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\*2. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.3. \*\*Called to Linda S., RN L8 @ 1300 7/7/031. Review **Culture Summary** for accuracy before filing report.
2. Continued reports: If there are more isolates to report, than lines in Sunquest MRE, it will be necessary to create a continued report. In Order Entry, order WDCC (Wound Culture Continued Report), using the same date/time. Add “SEEC” to the original accession and “RCON” to the new accession. It will be necessary to free text the new and old accessions after the SEEC and RCON comments. Refer to MCVI 5.0 Micro/Viro Computer for complete details.
3. 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 policy MCVI 5.1 LABELINGERRORS/SPECIMEN MIXUP for Sunquest report entry information.

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.* Refinal the culture when identifications and/or testing are complete.
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| **References** | 1. York, M., Section 3, Aerobic bacteriology, 3.13, Garcia, Lynne (ed) *Clinical Microbiology Procedures Handbook*, 2010, American Society for Microbiology, Washington, D.C.
2. Pezzlo, M., Section 2. Aerobic bacteriology, 2.1 and 2.10. *In* H.D. Isenberg (ed) *Essential Procedures for Clinical Microbiology.* 1998, American Society for Microbiology, Washington, D.C., pg. 39-50, 102-110.
3. Forbes, B.A., et al., Bailey & Scott’s *Diagnostic Microbiology*, twelfth edition, 2007, Mosby, Inc., St. Louis, MO.
4. Pezzlo, M., Section 1, Aerobic bacteriology, 1.16, *In* H.D. Isenberg (ed) *Clinical Microbiology Procedures Handbook*, 1994, American Society for Microbiology, Washington, D.C.
5. Simor, A.E., F.J. Roberts, J.A. Smith, coordinating ed. J.A. Smith, *Cumitech 23, Infections of the skin and subcutaneous tissues,* 1988, American Society for Microbiology, Washington, D.C.
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| **Appendices** | BATTERY: WDCSPEC MEDIA0 AKV, ASB2, TSB, CHOC, SB, CNA, MAC, THIO, 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 | 1981 | Initial Version |
| 1.1 | Pat Ackerman | 12/27/1995 |  |
| 1.2 | Pat Ackerman | 08/14/2003 |  |
|  | 1.3 | Pat Ackerman | 09/05/2005 |  |  |  |
| 1.4 | Pat Ackerman | 01/18/2008 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. |
| 1.5 | Pat Ackerman | 11/14/2008 | Added EIP information |
| 1.6 | Tina Gronquist | 06/19/2014 | Updated into online format. |
| 2 | Becky Carlson | 4/18/2015 | Re- numbered from MC 435 for CMS load. |
|  | 3 | Susan DeMeyere | 9/7/2017 | Changed reporting to keep culture open while THIO incubates.  |