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| **Fungal Blood Culture/Fungal Bone Marrow Culture** |
| **Purpose** | This procedure provides instructions for Fungal Blood Culture/Fungal Bone Marrow Culture for the Microbiology laboratory. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and/or plate reading.  |
| **Principle and Clinical Significance** | Disseminated fungal infections are more prevalent than previously recognized, and blood culture is an important diagnostic tool. The Wampole Pediatric Isolator™ is a sensitive method for the recovery of dimorphic pathogens, yeasts and filamentous fungi. The Wampole Pediatric Isolator™ tube is intended for the collection of small volume blood specimens for isolation of microorganisms. The tube contains 0.96 ml sodium polyanetholesulfonate (SPS) as an anticoagulant and 0.1 ml of saponin to lyse red blood cells to release fungal cells without affecting the recovery of microorganisms. SPS also neutralizes the bactericidal properties of blood and inhibits phagocytosis. Upon receipt in the laboratory, the blood is removed and directly plated onto conventional media. |
| **Test Code** | **BCF, BMCF** |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram stain reagents
* Lactophenol cotton blue stain (LCB)
* Olive oil
* Vitek® YBC card
 | * 3 cc syringe
* BD™ Blood Transfer Device
* 70% alcohol pads
* blank discs (sterile)
* shrink seals
 | * Ambient air incubator 35ºC
* Incinerator
* Inoculating loop
* Microscope
* Vortex mixer
* MALDI
 | * Wampole Pediatric Isolator™ 1.5 ml microbial tube (ISL)
* Chocolate agar (CHOC)
* Sabouraud dextrose agar with chloramphenicol (SABC)
* BHI with 5% sheep blood (BBHI) – RT
* BHI with 5% sheep blood (BBHI) - 35ºC
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| **Sample** | 1. Acceptable specimens: blood, bone marrow
* SDES codes/Specimen type: 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: LUMEN Search option ○ Code ◙ Description Add to List Code Description  BLUL Blue Lumen REDL Red Lumen WHL White Lumen  Free Text…* Free text may be added to the specimen description code by clicking on Type free text in the text box and click OK. This will automatically append the free text on to the SDES code. Click on

 Select* ARL – Art line ● PERC – Perc line
* ARTP – Arterial puncture ● CORD – Cord blood
* BLDN – Blood, collect site not specified ● BLUL – Blue lumen
* BROV – Broviac ● WHL – White lumen
* CENL – Central venous line ● IVS – IV start
* HICK – Hickman ● UVC – UVC line
* MEDL – Medcomp line ● REDL – Red lumen
* PEBL – Peripheral blood ● FEM – Femoral
* PORT – Port-a-cath (PAC) ● CVP – CVP line
* UART – Umbilical arterial catheter (UAC) ● LD – Line draw
* PICL – PIC line ● BMAR—Bone Marrow
1. Specimen Collection and Transport
* Refer to [*Lab Test Directory - Fungal blood culture*](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033032.asp)
1. Specimen assessment
* Refer to the policy [*Specimen Rejection Criteria*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20100%20%20%20%20Quality%2CSpec.%20mgmt.%2CLabeling%2CProc.%2CSendout%20Results%2CBilling%2C%20PT%20testing%2CAddl%20Projects%5CMC%20103%20Specimen%20Rejection%20Criteria%20R.docx)*.*
1. Special instructions
2. Specimens should be processed as soon as possible after receipt resulting in faster isolation.
3. Specimens can be held up to 16 hours at room temperature without adverse effect on recovery of organisms.
4. Patients on antibiotic chemotherapy should be processed immediately.
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. [*Biohazard Containment*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment%20R.doc)
2. [*Safety in the Microbiology/Virology Laboratory*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20202%20Safety%20in%20the%20Microbiology%20Lab%20Policy%20R.docx)
3. [*Biohazardous Spills*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20204%20Biohazardous%20Spills%20R.docx)
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| **Storage** | Specimens can be held up to 16 hours at room temperature without adverse effect on recovery of organisms. |
| **Quality Control** | 1. Performance has been documented by Wampole Laboratories.
2. Record each new lot or shipment before put into service in QC manual.
3. For technical information contact Wampole technical services (800) 257-9525.
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| **Procedure** | InoculationWarm all media before inoculation. Label all plates properly with the patients name, accession number and date. Specimen processing1. Vortex the tube vigorously at the highest setting.
2. Disinfect the top of the stopper with 70% isopropyl alcohol. Allow to dry.
3. Attach a 3-ml syringe to the blood transfer device.
4. Push the tube into the holder of the blood transfer device and withdraw the contents.
5. Expel any air in the syringe into the tube and remove the tube from the transfer device.
6. Remove the blood transfer device from the syringe and discard the transfer device into the sharps container.
7. Divide the lysate equally among the primary plates, using a maximum of 0.35 ml per plate.
8. Keeping the lids of the plates as low as possible, dispense the blood in a straight line across the surface of the agar, avoiding the edge of the plate. Discard the syringe.
9. Cross-streak the inoculum evenly over the entire surface of the plate without flaming and avoiding the edges of the plate.
10. Place a sterile blank disc on the SAB (RT) and BBHI (35ºC) plates and add 1-2 drops of olive oil to each disc for the isolation of *Malassezia furfur.*

1. Wrap plates with shrink-seal. Allow to dry.
2. Incubation: Incubate plates in upright position for the first 24 hours to allow lysate to absorb into media. Invert plates after 24 hours.
* Incubate CHOC in CO2 at 35° C (in the Pos BC rack) for 2 days.
* Incubate SAB and BBHI at RT for 21 days.
* Place second BBHI with oil disc in ambient air incubator at 35ºC for 21 days.
1. Culture examination
2. Examine primary plates for visible growth at 24 and 48 hours. Invert plates after 24 hours.
3. 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.
4. If there is no visible growth on the CHOC, document result in a computer workup. Discard plate after 48 hours.

 Workups: Wkup # 1  Med : CHOC  Desc: ISL  Id: NG21. Continue to incubate SAB, BBHI (RT) and BBHI (35ºC) for 21 days. Examine for visible growth three times weekly (M-W-F). Check closely for growth around the oil discs.
2. Cultures exhibiting growth:
3. When growth appears, differentiate between bacteria, yeast or filamentous moulds. Perform Gram stain or stain with lactophenol cotton blue (LCB).
4. Set up definitive biochemical or identification procedures on all isolates with MALDI or Vitek Yeast ID card (YID).
5. Subculture filamentous moulds to SAB to be sent to MDH for definitive identification
6. Consult with physician regarding antimicrobial susceptibility testing on moulds or yeast.
7. Report preliminary *Aspergillus* results as presumptive—SUMP-ASPE.
8. Critical results must be telephoned to the physician or patient’s nurse immediately.
9. Additional Days

Complete identification procedures until all significant isolates are finished.Send updated report and finalize.Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 14 days in case a physician calls for further studies.1. Quantitative culture—(If requested).
2. The number of colony forming units (cfu) per ml of blood can be determined as follows:

CFU/ml = Total number of cfu, all plates X Number of plates inoculated Number of plates on which the blood volume organism would be expected to grow.2. Example: E. coliPlate # of colonies E. coli would be expected to grow on all platesBBHI (RT) 5 except SAB, therefore:BBHI (35C) 1SAB 0 12 CFU X 4 plates = 11 CFU/mlCHOC 6 3 plates 1.5 ml |
| **Interpretation/ Results** | 1. If a colony appears only within the area inoculated, it should be considered a positive culture regardless of genus or species.
2. While colony counts in pediatric blood cultures are generally higher than those found in adults, it is not uncommon for the counts to be <10 cfu/ml during episodes of bacteremia associated with upper respiratory tract infections or occurring after antibiotic therapy.
3. If colonies appear on both the inoculated area and outside the inoculated area, consider the colony within the inoculated area as a positive culture and the one outside the area as a contaminant.
4. If the colony appears only on the outside of the inoculated area, it should be considered a plate contaminant.
5. The clinical significance of an organism isolated from the blood should be determined by the physician, taking into consideration the patient’s clinical history, status, and repetitive cultures.
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| **Procedure Notes** | *Malassezia furfur*, a lipophilic normal skin yeast, has been associated with deep-line catheter-related systemic infections in neonates and infants receiving parental emulsion therapy. *M. furfur* requires a lipid supplement for growth such as olive oil. The colonies are cream to beige, are smooth to deeply folded and have a brittle texture that is difficult to suspend. |
| **Result Reporting** | 1. Critical Value: All positive blood cultures are reported immediately by telephone to the

 Physician or patient’s nurse. Document in the computer, the person called and the date/ time of the call.1. No growth cultures: Update NG cultures daily in *Microbiology Automatic No-Growth Result Entry* up to 21 days.

Add Enter worksheet BCF and BCF2 in worksheet box. Click Worksheet entry: BCF LAST UPDATE COMPLETED 07/07/2006 AT 1302Selected worksheetsBCF FUNGAL BLOOD CULTUREStart updateClick After 21 days, manually finalize the culture using the statement “No fungus isolated 21 days” (Sunquest code: **NF21**).1. Positive cultures: 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.

 Observations: 1. YEAST BEING ISOLATED AND IDENTIFIED 2. \*\*Called to DR. FUGATE AT 0830 8/30/033. Susceptibilities to follow. Workups: Wkup # 1  Med : CHOC  Desc: ISL  ID: NG2 Workups: Wkup # 1.1 Workup Components Med : SAB CAID : NEG Desc: WH SC : SAB ID: YEAS VID : 11. If the culture has a bacterial isolate, result the organism on line 1. On line 2, put the statement “No fungus isolated to date”, Sunquest MO code **NFTD**. Put notification information on line 3.

Observations: 1. ENTEROCOCCUS SPECIES ISOLATED. 2. NO FUNGUS ISOLATED TO DATE3. \*\*Called to L8 (JAN NELSON, RN) 0800 8/30/03Continue to hold fungal plates for 21 days. If the culture is negative for moulds and yeast after 21 days, finalize the culture using the statement “No fungus isolated 21 days” (Sunquest code: **NF21**).1. Susceptibility reporting and billing
2. Fungal susceptibilities are entered in the *Susceptibility* tab. In order to link to the correct AST battery; highlight the FMIC (Fungal MIC) keyboard from the drop down box. Highlight the organism for susceptibility entry. Enter susceptibility results with the appropriate interpretation. Refer to [MCVI 5.20 Micro SendOut Resulting](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.20%20Micro%20Sendout%20Resulting.docx)
3. Fungal Drug codes: Drug codes can be found in Function MIQ # 1.

 AMPT – Amphotericin B CASP – Caspofungin FCY5 – 5-Fluorocytosine FZOL – Fluconazole IZOL – Itraconazole KZOL – Ketoconazole POSA – Posaconazole VORI – Voriconazole1. Bill the susceptibilities on the *Billing* tab. Refer to [MCVI 5.31 Add on micro UM bill codes](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.31%20Add%20on%20micro%20UM%20bill%20codes.%202015.xlsx) for U of M or use

 FMT1 – Fungal MIC Texas drug 1 FMT2 – Fungal MIC Texas drug 2 FMT3 – Fungal MIC Texas drug 3 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.1. 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:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20100%20%20%20%20Quality%2CSpec.%20mgmt.%2CLabeling%2CProc.%2CSendout%20Results%2CBilling%2C%20PT%20testing%2CAddl%20Projects%5CMC%20102%20Labeling%20Errors%2C%20Specimen%20Mixups%2C%20Corrected%20Reports%20R.docx)
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| **References** | 1. Versalovic, James. et al, *Manual of Clinical Microbiology*, 2011, ASM press, American Society for Microbiology, Washington, D.C., pg. 1663-1664,1705.
2. Wampole package insert, circular IN-050C5-02, revised: April 1993, Wampole Laboratories, Div. Of Carter-Wallace, Inc, Cranbury, NJ 08512.
3. Macron, M., D.A. Powell and D.E. Durrell, Methods for optimal recovery of *Malassezia furfur* from blood culture, *J. Clinical Microbiology,* Nov. 1986, Vol. 24, No. 5, pg. 696-700.
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| **Appendices** | WORKLABEL MEDIA-FORM DEFINITIONBATTERY : BCF SPEC MEDIA ----------------------------------------------- 0 ISL, CHOC, SABC, BBHI, BBHI, OO BLD ISL, CHOC, SABC, BBHI, BBHI, OO BMAR ISL, CHOC, SABC, BBHI, BBHI, OO |
| **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 | Pat Ackerman | 10/25/93 | Initial Version |
| 1.1 | Pat Ackerman | 4/12/94  |  |
| 1.2 | Pat Ackerman | 8/29/03  |  |
|  | 1.3 | Pat Ackerman | 12/06/04  |  |  |  |
| 1.4 | Pat Ackerman | 12/10/05  |  |
| 1.5 | Pat Ackerman | 7/21/07  | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. |
| 1.6 | Becky Carlson | 06/05/08 | Added incubation conditions for CHOC agar. |
| 1.7 | Becky Carlson | 04/17/09  | Added Bone Marrow to title box and in Acceptable specimens section |
| 1.8 | Becky Carlson | 1/05/2014 | Revised positive culture isolates retention time to 14 days |
| 1.9 | Tina Gronquist | 1/6/2014 | Reformatted into online format |
| 2 | Becky Carlson | 4/14/2015 | Re-numbered from MC405 for CMS |
| 3 | Susan DeMeyere | 9/19/2017 | Added to result as NF21 and link to billing codes and send out reporting.  |
| **Archived by:** |  | **Archived Date:** |  |