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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 plate reading. |
| **Principle and Clinical Significance** | The Bactec Myco/F Lytic Bottle is designed for the rapid detection of yeast and fungus in blood. Specimens are inoculated into the BD Bactec Myco/F Lytic vial either by syringe or direct draw with a needle and tubing. The vial is placed into the BD Bactec fluorescent series instrument and is continuously agitated and incubated at 35 ºC for maximum recovery. The recommended testing protocol is 7 days for yeast and 30 days for fungi. Each vial contains a sensor which can detect decreases in oxygen concentration in the vial resulting from microorganism’s metabolism and growth. The sensor is monitored by the BD Bactec fluorescent series instrument every 10 minutes. Analysis of the rate of oxygen decrease as measured by increasing fluorescence enable the BD Bactec fluorescent series instrument to determine if the vial is instrument positive. A positive determination indicated the presumptive presence of viable microorganisms in the vial. Detection is limited to microorganisms that will grow at 35ºC. The medium is not selective and will support the growth of other aerobic organisms.  |
| **Test Code** | BCFBMCF |
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| **Materials** | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram stain reagents
* Lactophenol cotton blue stain (LCB)
* Acridine Orange Stain
* Olive oil
 | * 1 cc syringe
* BD™ Blood Transfer Device
* 70% alcohol pads
* Blank discs (sterile)
* Shrink seals
 | * BACTEC™ FX - Analyses of the decrease of O2 concentration that enables the instrument to determine if the vial is positive.
* Ambient air incubator 35ºC
* Incinerator
* Inoculating loop
* Microscope
* VITEK MS
* VITEK 2
 | * Bactec Myco /F Lytic Bottle (red bottle)

Each vial contains:* 40 mL Processed water
* 0.07% 7H9 Middlebrook Broth Base
* 0.5% Brain Heart Infusion
* 0.1 Casein Hydrolysate
* 0.1% Supplement H
* 0.025% SPS
* CO2 and O2
* 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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| Storage**Sample** | Store bottles at 2-25ºC in a dry location out of direct light.* Acceptable specimens:
* Blood: inject 1-5 mL. Optimum amount is 3-5 mL.
* Bone marrow: inject 1-5 mL. Optimum amount is 3-5 mL.
* For specimen collection, transport, storage and rejection information, refer to the Lab Test Directory:
* Lab Test Directory [Blood Culture, Fungus](https://www.childrensmn.org/References/Lab/microbioviral/blood-culture-fungus.pdf)
* Lab Test Directory [Bone Marrow Culture, Fungus](https://www.childrensmn.org/References/Lab/microbioviral/bone-marrow-culture-fungus.pdf)

 * *Special instructions*
1. The BD Bactec vial should be transported as quickly as possible to the laboratory and placed into the BD Bactec instrument.
2. DO NOT USE culture bottles that exhibit any cracks or defects; discard the vial in the appropriate manner.
3. Do not use bottles showing evidence of contamination such as cloudiness, bulging, or depressed septum or leakage.
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. [*Biohazard Containment*](https://starnet.childrenshc.org/References/labsop/mcvi/safety/mcvi-3.1-biohazard-containment.pdf)
2. *Safety in the Microbiology Laboratory*
3. *Biohazardous Spills*
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|  **Quality Control** | **Media Quality Control**Commercially prepared blood culture media do not require additional in-laboratory QC per CLIA and CLSI M22-A3.Each case of media has a Quality Control certificate from BD indicating the organisms tested and the acceptability of those tests. An example from each media type is kept on file. |
| **Procedure** | **Entering Data and Loading the Instrument**1. To enter vials in the instrument, select a drawer using the indicator where there are available stations.
2. Do not select a drawer with the blue dot in the white circle. Select a different drawer.
* The blue dot indicates the instrument is reading. Pick a drawer that does not have a blue dot in the white circle.

1. Then follow one of the two methods described below.

Method 1 (Vial Activated)1. Select a drawer that has available stations. Use the indicator on the front of the drawer that indicates which drawer is currently in use.
2. Check if the drawer is currently reading bottles by looking for the blue dot in the white circle (see yellow arrow). Select a different drawer without the blue dot and open that drawer.

3. The barcode scanner turns on.4. Scan a vial sequence barcode label and the Accession barcode. 5. The Vial Entry display appears and the Sequence, Media, and default Protocol are automatically entered.6. If you did not scan the Accession, scan or enter it now.7. To change the protocol tap the “modify” button, then tap the up arrow to increase or down arrow to decrease the protocol length.8. Place the vial into an available station (solid green indicator)Method 2 (Icon Activated)1. Select a drawer that has available stations. Use the indicator on the front of the drawer that indicates which drawer is currently in use.
2. Check if the drawer is currently reading bottles by looking for the blue dot in the white circle (see yellow arrow). Select a different drawer without the blue dot and open that drawer.
3. Tap the “vial entry” button on the Status display.
4. The Vial Entry display appears and the barcode scanner turns on
5. Scan the vial sequence barcode label
6. The Sequence, Media, and default Protocol are automatically entered
7. Scan the Accession barcode.
8. To change the protocol tap the “modify” button, then tap the up arrow to increase or down arrow to decrease the protocol length
9. Place the vial into an available station (solid green indicator)
10. When a vial is placed into the last available station in a drawer, the Activity Complete tone sounds (3 beeps).
11. To continue entering vials, select another drawer with available stations.
	* + 1. **Inserting Vials in the Instrument**
			2. Before inserting vials into the stations, visually inspect all vials for growth. Evidence of microbial growth includes hemolytic, turbidity, and excess gas pressure (causing the vial septum to bulge outward). All such vials should be treated as positives; they should be stained and subcultured.
			3. After all vials have been inspected and inserted in stations, close the drawer.
			4. A vial presence sensor immediately senses the insertion of a vial in a station and the instrument updates the station LED indication and the status shown on the LCD.
			5. Once vials are placed in their stations, you should avoid moving them to other stations unnecessarily.
			6. Avoid opening the drawer unnecessarily. Drawers should not remain open longer than 10 minutes. Continuous loud alarm will sound if drawer is open more than 10 minutes.
			7. Make sure all vials are fully inserted in the stations before closing the drawer.

Vials that are not read for 40 minutes (because of an open drawer or being unseated) need to be subbed and an AO performed. If AO stain is positive, perform Gram stain.* + - 1. **Vials Delayed in Transport—**(add **DELA** to the **SDES** when receiving these cultures).

Vials that are delayed in transportation to lab 8 hours or more need to be subbed to CHOC, SABC, 2 BBHI plates, and a Gram stain and AO performed before being placed into Bactec. * + - * 1. Subculture the bottle(s) according to the positive bottle BCF protocol.
				2. Read the preliminary Gram stain. Leave results for day shift Micro with a label indicating the status.
				3. If the Gram stain is negative, put the bottle(s) in the Bactec according to the processing new vials protocol.

Bottles can be held up to 48 hours at room temperature and up to 12 hours in a 35-degree incubator, and still be placed into the Bactec for reading.* + - 1. **Anonymous Vial Entry**
			2. Vials can be placed into available (GREEN indicator) stations without being scanned into the instrument. Vials that are not scanned into the instrument are called “anonymous” vials. The instrument recognizes anonymous vials when they are placed in stations, but are assigned an “unknown” medium type and default protocol of 5 days. Anonymous vials are evaluated with general positivity criteria. They cannot use the specific positivity criteria tied to the characteristics of the medium since the instrument does not know the medium type.
			3. These anonymous vials need to be identified in the system using the ID(entify). Do not perform Negative Vial Removal until all Anonymous vials have been resolved. You could lose data if you accidentally remove an Anonymous vial.
			4. To identify anonymous vials:
1. Open drawer and remove vial from flashing yellow station or open drawer and tap ? to activate ID Anonymous workflow.
2. Scan the sequence and accession for the anonymous vial. The patient information is filled on the workflow display and the station the vial was pulled from will be flashing green.
3. Return the vial to the flashing green station.
	* + 1. NOTE: Once an anonymous vial has been placed in the instrument, do not remove the vial and reenter it without identifying it (ID Anonymous activity). All test readings are discarded if you remove the vial without identifying it.

**Positive / Negative/ Ongoing Vials** 1. **Notification of positive and negative vials**
	1. The system notifies you of new positive cultures in several ways
		1. Positive Vial audible alarm sounds
		2. Station Indicators: FLASHING RED or FLASHING AMBER / RED (alternating) -Anonymous Positive
		3. Message box appears on Epicenter screen.
		4. Positive vial system indicator for that drawer illuminates
		5. On the Status display, the “positives” icon is active (color is red, not grayed out) and the number of positive vials in the drawer is shown
			1. Out-of-Protocol Negatives are indicated by the following
				1. Negative vial system indicator for that drawer illuminates
				2. On the Status display, the “negatives” icon is active and the number of negative vials in the drawer is shown
				3. Station indicators: FLASHING GREEN

In Protocol Negatives (ongoing) are indicated by LED with no light lit up.**Removing positive vials**1. Print “**Current positive** report”. At the FX screen, touch the **Reports** tab. Touch the drop-down menu and select **Current positives.** Touch the **Print** button at the bottom of the screen.
2. Select a drawer that has positive stations, and open the drawer by pulling it out.

The barcode scanner turns on. All positive, final negative, available, and anonymous (all variations) are indicated by the appropriate lit or flashing station indicators.Tap the “remove positives” button on the Status display, OR Remove a vial from a FLASHING RED (positive) or FLASHING AMBER / FLASHING RED (anonymous-positive) station3. The Positive Removal display appears. Scan vial sequence. (If an anonymous positive vial was removed, the ID Anonymous display appears. Scan the sequence and accession for the anonymous positive vial and tap the “Save” button. Then tap the “Exit” button to return to the Positive Removal display.* + - * 1. **Negative Vial Removal**

Negative bottles will be removed at the beginning of each shift. Open drawer.Remove negative vial from Flashing Green station (Vial activated workflow). OR:Tap the “remove negatives” button to activate Negative Removal Workflow (Icon Activated workflow).“Remove Negative” Workflow display is activated.Only negative vial station LEDs are illuminated flashing green and the barcode reader is not turned on.Continue removing negative vials until all vials with flashing green LEDs are removed.Dispose of bottles in biohazard waste containers. Retain bottles labeled with white tape “flag” labeled TSUB. Follow False Positive Bottle instructions.If a completed Out-of-Protocol vial is accidentally left in the instrument, it will remain negative and can be removed at a later time.Triple beep (workflow complete) will sound.**Processing an Instrument – Positive Vial**Remove the vial from the instrument and place in a biological safety cabinet.Reprint the specimen label to use on the subculture plates.If it is necessary to release pressure in the vial, place a 70% isopropyl alcohol wipe over the septum and insert a venting needle through the alcohol wipe and septum. Remove the needle after the pressure is released. Place the venting needle into a sharps container.Invert the vial to mix the contents.Disinfect the septum of the vial with a 70% isopropyl alcohol wipe. Allow to dry.Attach a 1 ml syringe to a blood transfer device.Push the blood transfer device into the septum of the vial, invert and withdraw 1ml.Remove the blood transfer device with the syringe from the vial.Remove the syringe from the blood transfer device and discard the blood transfer device into a sharps container.Using the contents from the syringe, inoculate a CHOC, SABC, 2 BBHI, from the Myco/F Lytic bottle to streak for isolation.  * Label the plates with the current date, the current time, mark them “**F**” for the Myco/ F Lytic bottle (use the barcode labels).
* Place 1 drop of blood in each plate and streak for isolation.
* Place a sterile blank disc in the first quadrant, on the SABC (RT) and BBHI (35ºC) plates and add 1-2 drops of olive oil to each disc for the isolation of *Malassezia furfur.*
* Wrap plates with shrink-seal. Allow to dry.
* Incubate the Chocolate plate for 2 days in CO2, to screen for aerobic organisms.
* Incubate the SABC with oil disc and BBHI at RT.
* Incubate the BBHI with oil disc in ambient air incubator at 35ºC.

Make a Gram stain slide.Expel the remaining sample into a sterile and labeled snap cap tube.After the slide is dry and heat fixed, perform the Gram stain procedure as soon as possible.Read and report the Gram stain results. (See Reporting section).16. If no organisms are seen, refer to False Positive Bottle section.17. **Day shift:** Perform an AO if Gram stain is negative.18. Perform identification and susceptibility (AST) of organism(s) grown on solid media according to laboratory protocol.19. Positive bottles are saved for one month in case of additional testing.E. **Incubation**: 1. Incubate CHOC in CO2 at 35° C (in the Positive BC rack) for 2 days.
2. Incubate SABC and BBHI at room temperature (RT BCF rack).
3. Incubate the second BBHI with oil disc in ambient air incubator at 35ºC (BCF or BC rack).

**FALSE POSITIVE BOTTLE**1. If no organisms are seen on Gram Stain, mark the **False Positive** (Gram stain negative) bottles with a tape “flag” labeled **TSUB**. This will alert day shift techs for AO stain and terminal subculture.
2. Return the flagged bottle to the Bactec. Save sample in snap cap for day staff for AO testing.
3. Do not call results to provider.
4. Day shift: When the False Positive bottle becomes a Bactec out of protocol negative at 30 days:
5. Perform terminal subculture (TSUB): Inoculate CHOC and SABC.
6. Label the plates with the current date, the current time, mark them “F” (use the barcode labels) and incubate.
7. Perform AO stain from these TSUB bottles.
8. Examine plates for 7 days before discarding as negative.
9. **Culture examination**
10. Examine primary plates for visible growth at 24 and 48 hours. If growth is not visible, hold plates for 7 days.
11. 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.
12. Cultures exhibiting growth:
13. When growth appears, differentiate between bacteria, yeast or filamentous molds. Perform Gram stain or stain with Lactophenol cotton blue (LCB).
14. Set up definitive biochemical or identification procedures on bacterial or yeast isolates (i.e., VITEK MS or VITEK 2).
15. Subculture filamentous molds to SAB to be sent to MDH for definitive identification.
16. Report preliminary *Aspergillus* results as presumptive (Sunquest codes: SUMP-ASPE) and send to MDH for definitive identification.
17. Consult with physician regarding antimicrobial susceptibility testing on molds or yeast.
18. **Critical results must be telephoned to the physician or patient’s nurse immediately.**
19. 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.
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| **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** | * BCF cultures will be held for 30 days in the Bactec Instrument.
* *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.
* **Contamination-**Care must be taken to prevent contamination of the sample during collection and inoculation into the Bactec™ vials. A contaminated sample will give a positive reading, but this does not indicate a clinically significant result. Such a determination must be made by the user, based on such factors as type of organisms recovered, occurrence of the same organism in multiple cultures, patient history, etc.
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| **Critical Values** | Critical Value: Isolation of following will be reported immediately by telephone to the physician or the patient’s nurse. Document in the computer the person called and the date/time of the call.* Mucorales-all genera included in this Order, including those listed below
* *Mucor spp.*
* *Rhizomucor spp.*
* *Rhizopus sp.*
* *Syncephalastrum spp.*
* *Lichtheimia spp.*
* *Cunninghamella spp.*
* *Cryptococcus neoformans*
* *Coccidioides immitis*
* *Histoplasma capsulatum*
* *Blastomyces dermatitidis*
* *Sporothrix schenkii*
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| **Result Reporting** | 1. Critical Value: All positive 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.* Record ALL results on the Bactec “Current Positive” print-out.
* Record F for the Myco F Lytic bottle.
* Record/write the Gram results.
* Record the “Called to”, with date and time.
* Record tech initials
1. **No growth** cultures: Desk 2 will update NG cultures daily in *Microbiology Automatic No-Growth Result Entry*
* Enter worksheet BCF in worksheet box.
* Click ‘Add’.
* Click ‘Start Update’.
* After 30 days, BCF cultures will automatically finalize as “No fungus isolated 30 days”
1. **Positive cultures**: Record results in Sunquest MRE in the Culture Entry tab and Workup section. Use code BRED for the red bottle. An example is as follows:

 Observations: 1. YEAST BEING ISOLATED AND IDENTIFIED 2. \*\*Called to DR. FUGATE AT 0830 8/30/033. Susceptibilities to follow. Workups: Workup # 1 Workup Components Med : BRED Gram: Yeast  Desc: Pos  ID: UNKN Workups: Workup # 1.1 Workup Components Med : SABC MSID: 1 Desc: WH SC: SAB ID: YEAS Gram: Yeast1. 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 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. If the culture is negative for molds and yeast after 30 days, finalize the culture using the statement “No fungus isolated 30 days” (Sunquest code: **NF30**).1. Susceptibility reporting and billing:
* Fungal susceptibilities are entered in the *Susceptibility* tab. Refer to [MCVI 5.20 Micro Send-Out Reporting](https://starnet.childrenshc.org/References/labsop/mcvi/comp/mcvi-5.2-micro-sendout-resulting.pdf) for instruction.
* 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 send-outs.

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.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 [MCVI 5.1 Mislabeled & Unlabeled Specimens](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.1%20Mislabeled%20%26%20Unlabeled%20Specimens.docx)
 |
| **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. 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.
3. BD BACTEC Myco/F Lytic Culture Vials F013041 (02) 2023-05 Becton, Dickinson and Company 7 Loveton Circle Sparks, Maryland 21152 USA
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| **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.  |
| 3 | Susan DeMeyere | 10/22/2018  | Biennial review  |
| 4 | Susan DeMeyere | 12/18/2023 | Updated to BD BACTEC Myco/F Lytic bottles, discontinue Wampole Isolator tubes.  |