TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	10/1/2015
Department:	Core	**Due Date:**	10/28/2015
		Implementation:	**10/28/2015**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Blood Culture, with Automated Detection SGAH.M17,WAH.M17 v3

Blood Culture Gram Stain Referral / Consult Form AG.F335.0

Description of change(s):

SOP

Section	Reason
8.3 C.8	Add using loop to spread smear
8.3 C.12	Add Previ stainer
8.3 D.1	Add to scan the patient bar code first
8.3 D.4	Add completion of worksheet
8.3.D.5	Edit to file slides in slide box
8.3.D.6	Edited times for aerobic and anaerobic plates
8.3.D.9	Added instructions for NOS bottle detected a second time
10.6	Added M05 SOP to consolidate
16	Added ROB, FES, Video Microscope & Referral form
19	Added keyboard and flow chart

Notes:

Blood Culture; Positive Workup SOP will be **discontinued**. Content has been added to the above SOP (*mostly to section 10.6 and addenda*)

The Blood Culture Gram Stain Referral / Consult Form will be placed into document control (proper header and form # added to bottom). The content did NOT change.

Revised SOP & FORM will be implemented on October 28, 2015

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training (version 3)

Technical SOP

Title	**Blood Culture, with Automated Detection**
Prepared by	Leslie Barrett Date: 8/13/2009
Owner	Ron Master Date: 8/13/2009

Laboratory Approval	**Local Effective Date:**	
Print Name	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Form revised 10/31/02

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Culture, Blood	BACTEC™ 9240 Continuous Monitoring Fluorescent System	XBLC

Synonyms/Abbreviations
Blood culture, BACTEC™, Routine Blood Culture

Department
Microbiology

2. ANALYTICAL PRINCIPLE

The BACTEC™ 9240 instrument is designed for the rapid detection of microorganisms in blood. Blood samples are drawn from patients and injected directly into BACTEC™ culture bottles. These bottles are then entered into the BACTEC™ 9240 for incubation and continuous automated monitoring. Microorganisms in the blood culture bottle, if present, will metabolize nutrients in the culture medium, which results in the release of carbon dioxide (CO_2). The CO_2 reacts with a dye in the sensor that is located in the pad at the bottom of each bottle. This sensor modulates the amount of light that is absorbed by the fluorescent material. Photo detectors positioned in the BACTEC™ instrument measure the level of fluorescence every ten minutes. The fluorescence intensity in the pad is directly proportional to the amount of CO_2 present in the blood culture bottle. The fluorescent units measured by the BACTEC™ instrument are interpreted according to preprogrammed positivity parameters; a positive reading indicates the presumptive presence of viable microorganisms in the blood culture bottle.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	**Collection:** Prior to inoculation, the broth media in the bottles should be clear. Do not use bottles containing broth that is cloudy. It is critical that blood specimens submitted for culture are collected aseptically. Contamination of specimen with skin flora can result in a false positive culture, which may be difficult to interpret clinically and lead to unnecessary antimicrobial therapy. Please refer to Blood Culture Protocol, Phlebotomy for specific instructions related to specimen collection and the inoculation of bottles. **Timing:** Before administering systemic antimicrobials, the collection of 2 separate sets of blood cultures is recommended when there is a fever combined with significant leukocytosis or leukopenia. Recommendations are as follows: **Systemic and localized infections** a. Suspected acute sepsis, meningitis, osteomyelitis, arthritis, or acute, untreated bacterial pneumonia: Obtain 2 sets of blood cultures from separate sites before starting antimicrobial therapy.

Component	Special Notations
Specimen Collection and/or Timing (con't)	b. Fever of unknown origin: obtain 2 sets of blood cultures initially and 1-2 additional sets 24-36 hours later. Note: The yield beyond 4 sets of blood cultures is negligible. c. Suspected early typhoid fever or brucellosis: owing to the low grade bacteremia present in these infections; obtain 4 sets of blood cultures over a 24-36 hour period. **Infective endocarditis** a. Acute: obtain 3 sets of blood cultures during the first 1 – 2 hours of evaluation. b. Subacute: obtain 3 sets of blood cultures on the first day (ideally, 15 or more minutes apart; the same venipuncture site may be used). If all 3 sets are negative, obtain 2 additional sets of cultures.

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Blood specimens inoculated into BACTEC™ PLUS Aerobic/F and BACTEC™ PLUS Anaerobic/F or BACTEC™ PEDS PLUS/F bottles.
-Other Acceptable	None
Collection Container	BACTEC™ PLUS Aerobic/F or BACTEC™ PLUS Anaerobic/F or BACTEC™ PEDS PLUS/F bottles.
Optimum Recommended Volume per BACTEC™ Bottle	**Neonates and Children 1 to 6 years:** In BACTEC™ PEDS PLUS/F bottles: 1.0 to 3.0 mL. blood/bottle. **Adults:** In BACTEC™ PLUS Aerobic/F and BACTEC™ PLUS Anaerobic/F: 8 to10 mL blood/bottle.
Minimum Volume per Bottle	BACTEC™ PEDS PLUS/F bottles: 0.5 mL blood/bottle is acceptable, but 1mL is preferred. BACTEC™ PLUS Aerobic/F and BACTEC™ PLUS Anaerobic/F: 3 mL blood/bottle is acceptable but not recommended.
Transport Container & Temperature	Same as collection container, at room temperature.
Stability & Storage Requirements	Store inoculated bottles at room temperature. Do not refrigerate or freeze, and do not pre-incubate bottles prior to shipment. Bottles are stable for up to 48 hours after collection at room temperature. If bottle(s) are received beyond these stated limits, they may be rejected, notify a supervisor.

Criteria	
Timing Considerations	N/A
Sub-Optimal & Unacceptable Specimens & Actions to Take	• Blood cultures submitted in expired or refrigerated BACTEC™ bottles. • Blood cultures submitted in any other tube, container, etc. • Reject the specimen and request recollection.
Compromising Physical Characteristics	None
Other Considerations	N/A

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety information. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
BACTEC ™ Plus Aerobic/F Medium	BD, Cat. # 442192, SC #64452
BACTEC ™ Plus Anaerobic/F Medium	BD, Cat. # 442193
BACTEC ™ Peds Plus Medium	BD, Cat. # 442194, SC #114693

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	All BACTEC™ bottles listed in above table
Container	N/A
Storage	Store at 2-25°C in a dry location and out of direct sunlight.
Stability	Stable until stated expiration date.
Preparation	Ready for use.

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Each case of media has a manufacturer's Quality Control certificate indicating the organisms tested and the acceptability of those tests. These certificates must be maintained as quality assurance/quality control documentation.

6.2 Control Preparation and Storage

N/A

6.3 Frequency

N/A

6.4 Tolerance Limits

N/A

6.5 Review Patient Data

Review patient results for unusual patterns, trends or distributions in patient results. Those would include: an unusually high percentage of positive or negative culture results, a high number of false positive bottles, or a high recovery rate of an unusual organism. Computer aided tools should be used when available.

6.6 Documentation

N/A

6.7 Quality Assurance Program

The laboratory participates in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

BACTEC™ 9240 Blood Culture System

7.2 Equipment

1. BACTEC™ 9240 Fluorescent Series Instrument
2. BACTEC™ Computer and peripherals
3. BACTEC™ Bar code scanner
4. Class II Biological Safety Cabinet (BSC)
5. Incubator, 35 ± 2 ºC, with 5-10% CO_2
6. Bacti-cinerator or equivalent (optional)
7. Slide Warmer (optional)

7.3 Supplies

1. BACTEC™ Vial/Thermometer
2. Safety SubCulture Unit
3. Disposable Sterile Inoculating loops
4. Glass microscope slides
5. Alcohol wipes
6. BACTEC™ station blocker for vial/thermometer placement
7. 0.5 McFarland Standard
8. Anaerobic Gas Generator Packs

8. PROCEDURE

NOTE: Manipulations of bottles such as subculture and preparation of smears must be performed in a BSC. Refer to safety section 15.0 for specific information regarding blood culture bottles. Report all accidents to a supervisor.

The package insert for a new lot of bottles must be reviewed for any changes before the media is released for distribution. A current Package Insert is included as a Related Document.

8.1	Instrument Set-up Protocol
A	**DAILY MAINTENANCE:** The following procedures are performed at the start of each day's testing and recorded on the BACTEC Maintenance Log
1.	Check printer's supply of paper. If paper supply is low or exhausted, replace. Refer to BACTEC ™ Fluorescent Series Users' Manual.
2.	Check temperature readout of each rack and cabinet air on the instrument's temperature controller. Verify each rack is currently at 35°C ± 1.5 ° C and the cabinet temperature is at 30° C ± 1.0° C. Also verify that the calibrated internal temperature probe is at 35° C ± 1.5 °C. If any rack or cabinet is not within temperature range, call BD Field Service. Record data on the BACTEC Maintenance Log.
3.	Check rack indicator operation by opening the instrument door and using the barcode scanner. a) Scan the selection, **ILLUMINATE GREEN RACK INDICATORS**. Listen for a beep indicating a successful scan. The GREEN lamps at each station should illuminate. If any lamp does not, the station should be removed from service and plugged to prevent its use until repaired. Refer to BACTEC ™ Fluorescent Series Users' Manual b) Scan the selection, **ILLUMINATE RED RACK INDICATORS**. Listen for a beep indicating a successful scan. The RED lamps at each station should illuminate. If any lamp does not, the station should be removed from service and plugged to prevent its use until repaired. Refer to User's Manual. c) Scan the selection, "Illuminate FRONT panel indicators." All four of the indicator lamps on the front of the instrument should illuminate together then one at a time. If

	any lamp does not, refer to user's manual (section 6.7) for instructions on replacing the burned out lamp. d) Scan the selection, "Audible alarm test." The instrument's audible alarm will sound three times. The pattern "BD" is also displayed in the station LEDs (light emitting diodes) followed by the instrument number. If the alarm doesn't sound, contact BD Field Service.
4.	Perform system backup using a 3.5 inch, high density, formatted diskette: a) From the main menu, press **[F5]** or Utilities Menu. b) From the Utilities menu, press **[F5]** or Backup. c) Insert the diskette into the disk drive. d) Press **[F10]** to begin the backup process. e) Once the backup is complete, remove the diskette and store in a safe place. Use a separate diskette for each day of the week (maintain a set of seven diskettes, labeled with each day of the week). Overwrite the previous Monday's data with the current Monday, the previous Tuesday's with the current Tuesday, etc.
5.	Print a 24 Hour Vial Inventory Report a) Press **[F7]** Reports b) Type"Y" in front of Hour Vial Inventory Report line c) Press **[F10]** d) Review the report for missing patient name or collection date/time e) Missing data must be resolved.
B	**WEEKLY MAINTENANCE:** Check the air filter at the rear of the instrument. Clean and/or replace the filter as needed. Refer to BACTEC ™ Fluorescent Series Users' Manual. Record on the BACTEC Maintenance Log.

8.2	**Test Run**
1.	**LOADING INSTRUMENT:** Blood culture bottles must be placed onto the instrument as soon as possible after receipt into the laboratory. Blood culture bottles will be entered into the BACTEC throughout the day, evening and night shifts. The BACTEC should be checked at least hourly for positive cultures. Refer to BACTEC™ Fluorescent Series Users' Manual for identification of anonymous bottles.
2.	Observe rubber septa of bottles. If residual iodine is apparent, remove with 70% isopropyl alcohol prior to loading the instrument. Make sure bottom of bottle is not obscured in any way by a barcode label or tape.
3.	Arrange bottles in sets.
4.	Open doors to instrument currently being used.
5.	Use bar code scanner on the right door to scan ***patient bar code first*** followed by ***BACTEC bar code.*** (If a patient bar code is not available on the bottle, scan the "Accession Not Available" bar code on the inside of the right door of the instrument).
6.	Place vial into station lit with red and green light.
7.	When all bottles have been entered, close instrument doors.

8.2	Test Run
	NOTES: • **Avoid placing bottles into the instrument without scanning the barcodes**. If bottles are not scanned into the instrument, they will become ANONYMOUS BOTTLES. An anonymous bottle must be identified as soon as possible so that the instrument can display the bottle's current status (i.e., ongoing, positive, etc.). • Scanning the **BACTEC** ™ bar code indicates to the instrument the medium type of the bottle that is being loaded so that the appropriate algorithms are utilized when the instrument reads the bottles. Without this information the instrument will still read the bottle, but will "hold" the information until the medium type is available. **This will delay "flagging" of a positive culture.**

8.3	**POSITIVE CULTURES:**
	A. Identification of Positives: The system will identify the presence of a positive culture by 1. The Positive Indicator Lamp on the front of the cabinet panel illuminates (yellow/orange). 2. An audible alarm sounds from the computer (press [F2] to silence). 3. On the computer's instrument status display the station number of the positive bottle(s) is displayed in flashing green, flashing red. The total number of positives will be displayed in the upper right hand corner of the monitor screen.
	B. Remove positive bottles: 1. Open instrument doors containing positive bottles, and using the instrument's barcode scanner, scan the menu option "Remove Positives". Listen for a beep indicating that the item was scanned successfully. 2. Locate a station with the FLASHING GREEN, FLASHING RED LEDs. 3. Remove the vial and scan its vial bar code only using the instrument bar code scanner. Listen for a beep and the LEDs will extinguish. 4. Repeat above steps to remove additional positive bottles until completed. 5. The acknowledged alarm condition is not clear until all the positive vials are removed.
	Perform the following steps in a Biological Safety Cabinet: C. Positive bottles: 1. Perform a Gram stain and subculture on each positive bottle. 2. Gently mix each of the positive bottles by gently inverting the bottles. 3. Visually inspect all positive blood cultures for gas. Use a Safety SubCulture Unit to vent the bottle. This will allow for equilibration of pressure prior to withdrawing liquid from the bottle. 4. Clean the top of each bottle with an alcohol wipe and allow to air dry. 5. Carefully open sterile package and remove the Safety SubCulture Unit.

8.3	**POSITIVE CULTURES:**
	6. Insert the Safety SubCulture Unit through the de-contaminated, clean, dry septum and remove the white filter cap. Do not discard the cap. 7. Tilt the bottle to dispense the inoculum onto each plate and slide. CAUTION: some bacteria can produce gas and the blood will pour out quickly rather than a drop at a time. Occasionally the Safety SubCulture Unit will become clogged. If this happens change the Safety SubCulture Unit. 8. Prepare a smear for Gram stain by placing a small drop onto a clean glass microscope slide. Use a loop to spread the drop to produce a thin, even smear. Allow the smear to dry completely. Heat fix slides prior to removal from BSC. 9. Subculture by placing several drops of broth onto the appropriate plates. Streak plates in order to achieve isolated colonies. All subculture plates should be incubated at 35±2°C. BAP and CHOC plates require 5-10% CO_2, and ANA BAP plates require anaerobic atmosphere. 10. Replace the white filter cap then remove and discard the Safety SubCulture Unit in a biohazard container. 11. Label plates with barcode labels (do not cover media type) and write the type of bottle (AER, ANA, or PEDS), date plated, and tech code near the bottom edge of the plate where it will not be covered by labels. Use a pencil to label a slide with accession number, patient last name, type of bottle (AER, ANA, or PEDS) and date positive. 12. See Gram Stain procedure for staining with Previ or Wescor Gram stainer and reporting gram stains. 13. See section 10.6 for reporting procedure.
	D. **SMEAR NEGATIVE (NOS)** bottles must be returned to the instrument. Carefully remove subculture device and dispose in biohazard waste. Bottles which have flagged as positive but have no organisms seen on Gram stain must be returned to their original positions on the instrument **within 3 hours**. NOTE: If the bottle is not returned to the instrument within 3 hours, the associated demographic data is removed from the database. The protocol for that bottle must be modified to reflect the remaining incubation time. Refer to the BACTEC ™ Fluorescent Series Users' Manual for instructions on vial re-entry. 1. Open instrument doors and scan the patient barcode then the vial's bar code using the instrument bar code scanner. The original station will be indicated by flashing green and red LEDs changing to solid green and red LEDs. 2. Place bottle in designated position. 3. Do not order XIDS or XIDSN and do not record the NOS Gram stain result in the LIS. 4. Print a workcard and label the subculture plates with barcode labels (do not cover media type) and write the type of bottle (AER, ANA, or PEDS), date and time plated, and tech code near the bottom edge of the plate where it will not be covered by labels. Complete the Positive Blood Culture Worksheet.

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8.3	**POSITIVE CULTURES:**
	5. Place the Positive Blood Culture Worksheet and plates together in the incubator in a biohazard bag. File the slides in the current Gram stain slide box. 6. Check plates at least once per shift for growth and record reading on the Positive Blood Culture Worksheet. Hold plates from smear negative aerobic bottles for 48 hours and 72 hours for anaerobic bottles if no growth. 7. If a bottle which has been returned to the BACTEC due to NOS is again flagged as positive by the instrument, a Gram stain and plating of the bottle must be performed again. 8. If growth detected on plates, perform a gram stain of the colonies and follow instructions in section 10.6. 9. If NOS bottle returned to instrument is detected as positive by the BACTEC a second time, a Gram stain and subculture of the bottle must be performed again.

8.4	**Remove Negative Bottles (batch) and Reporting Results**
1.	After 5 days of testing, bottles are identified as negative.
2.	Count the number of negative vials to be removed using the **Summary Window** on the right side of the computer screen.
3.	Open the doors of the BACTEC cabinet and scan "REMOVE NEGATIVES" bar code on the inside of the right door.
4.	The bottles which are negative will be indicated by a flashing green LED. Remove each bottle separately.
5.	Verify that the number of vials removed matches the number counted in the Summary Window. Close the doors.
6.	Use function MNG to report negative blood cultures.
7.	Negative cultures are reported as “No bacteria or yeast at 5 days” via MNG function once per day.

8.5	**Adding Patient Information to Vials Entered at Instrument**
1.	**Go to Culture Information screen by pressing the [ESC] key twice (2) followed by pressing [F3].**
2.	Press the [page down] key to advance to the Accession number field.
3.	Type the Accession number for the first set to be entered followed by pressing [F8]. Note: When this is done the vials attached to this accession number are displayed on the Vial information lines.
4.	Press [page up] key to return to Patient ID field.
5.	Type Patient ID followed by [enter]
6.	Type the Patient Name (last name, <space> first name) followed by [enter].
7.	Save the record by pressing the [F10] key.

8.6	**Resolving Anonymous Vials**
1.	Open doors to the instrument which contains the anonymous vial(s) and scan the ***"Identify Anonymous"*** bar code on the inside of the right door.
2.	The station that has the anonymous vial will light with a green light.
3.	Remove the vial from the station. Scan the **patient bar code first** followed by the ***BACTEC vial bar code.*** If a patient bar code is not on the vial, scan the "Accession Not Available" bar code on the inside of the right hand door.
4.	When the station changes from a green light to a red and green light, place the vial back into the station.
5.	Repeat steps 3 and 4 until you hear a three beep signal indicating that there are no more anonymous vials.
6.	Close the doors.

8.7	**When Vials are Placed into the Wrong Station**
1.	This usually results in the creation of an anonymous station and an error station.
2.	Open the doors to the instrument.
3.	Scan the "Identify Anonymous" bar code on the inside of the right door.
4.	Remove the vial from the station lit with the green light.
5.	Scan the patient bar code followed by the BACTEC vial bar code.
6.	If the computer monitor displays a message that says the vial scanned belongs in another station, **write down the station** that the message says the vial belongs in and press the [Esc] key on the keyboard.
7.	Place a BACTEC supplemental bar code label over the BACTEC vial label.
8.	Scan the patient bar code label on the vial followed by the BACTEC supplemental vial bar code.
9.	Place the vial in the station lit with the green **and** red light.
10.	Close the doors.
11.	Follow the procedure for changing status of a station to Manual Negative using the station written down in step 5.
12.	Leave a note on the instrument designating the location of the Manual Negative station just created so that it can be accounted for during the next batch negative removal.

8.8	**Changing Vial Status to manual Negative or manual Positive**
1.	At BACTEC computer, press [Esc] twice.
2.	Press [F3].
3.	[Tab] to station field.
4.	Type station. Press [F8].
5.	[Tab] to status field for this station. Press [F9].
6.	Type the letter "N" for manual Negative or "P" for Manual Positive. Press [F10] twice.

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

All data is interpreted by the instrument's computer system.

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Repeat Criteria and Resulting

N/A

10.6 Reporting in LIS

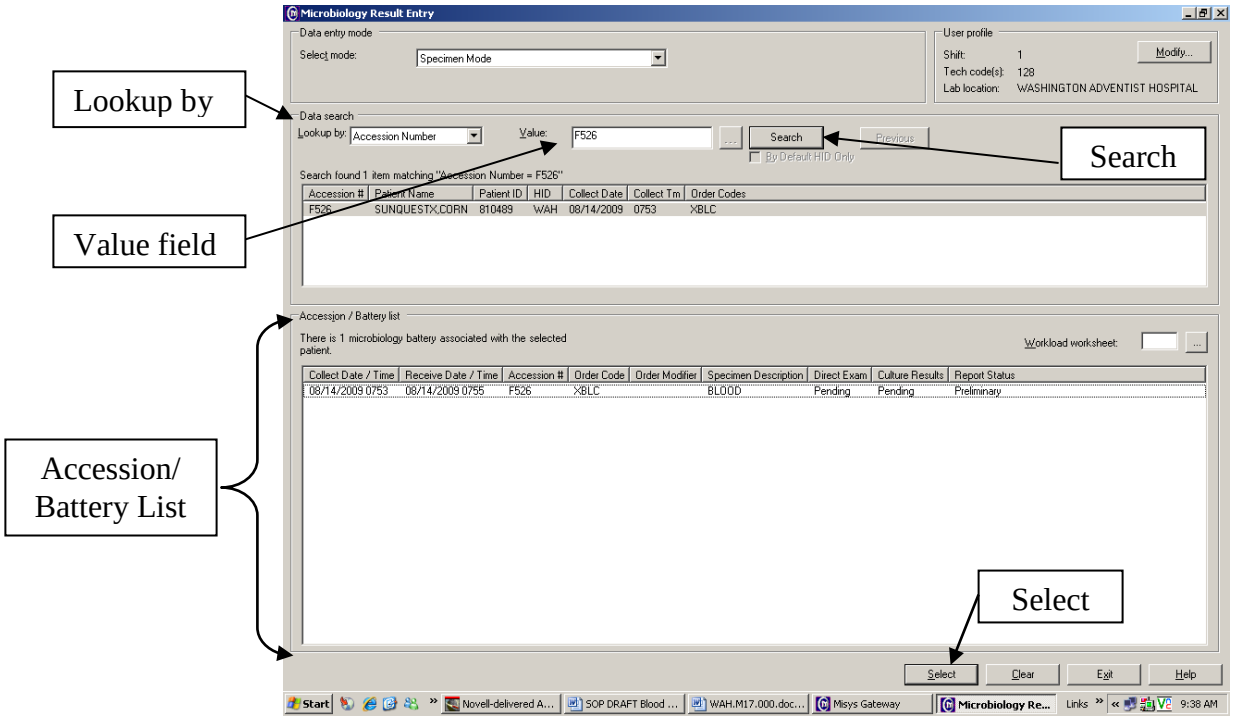
A. Blood Culture Order Sections

1. The Blood culture test code is XBLC
   Code XBLC consists of:
   - SDES - specimen description
   - SREQ - special request (This is usually a "HIDE" test, which doesn't display on reports unless a special request is added.)
   - IDST - ID & Sensitivity (This will be defaulted with "HIDE" as the result. It will be changed to "had been added" if the culture is positive.)
   - CULT - culture result (This will either be resulted as No Growth on the negative cultures or be resulted with the Gram Stain results on the positives)
   - RPT - report status (Pending, Preliminary, or Final)

2. The test code **does not include** an individual test code for gram stain.

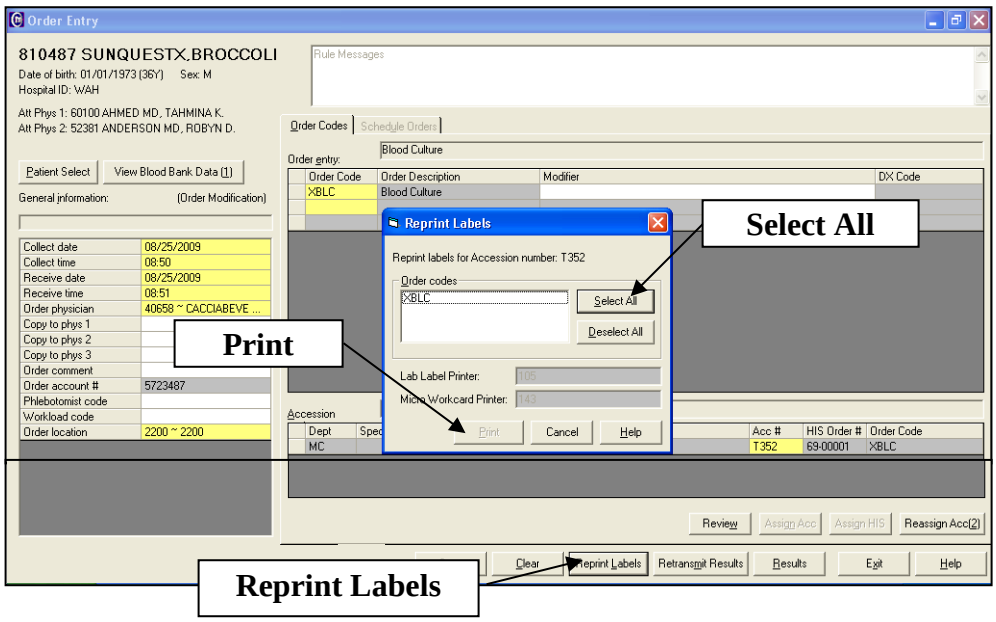
B. Positive Gram Stain: First Positive Bottle of a Set

1. Print the Work card
   a. Log on the Misys Gateway screen. Click on "Microbiology Result Entry" and enter the specimen accession number in the "Value" field. Press enter or click "Search". To search for the specimen by a different identifier, click the arrow for the drop down menu on the "Lookup by" field and search by name, medical record number, etc.



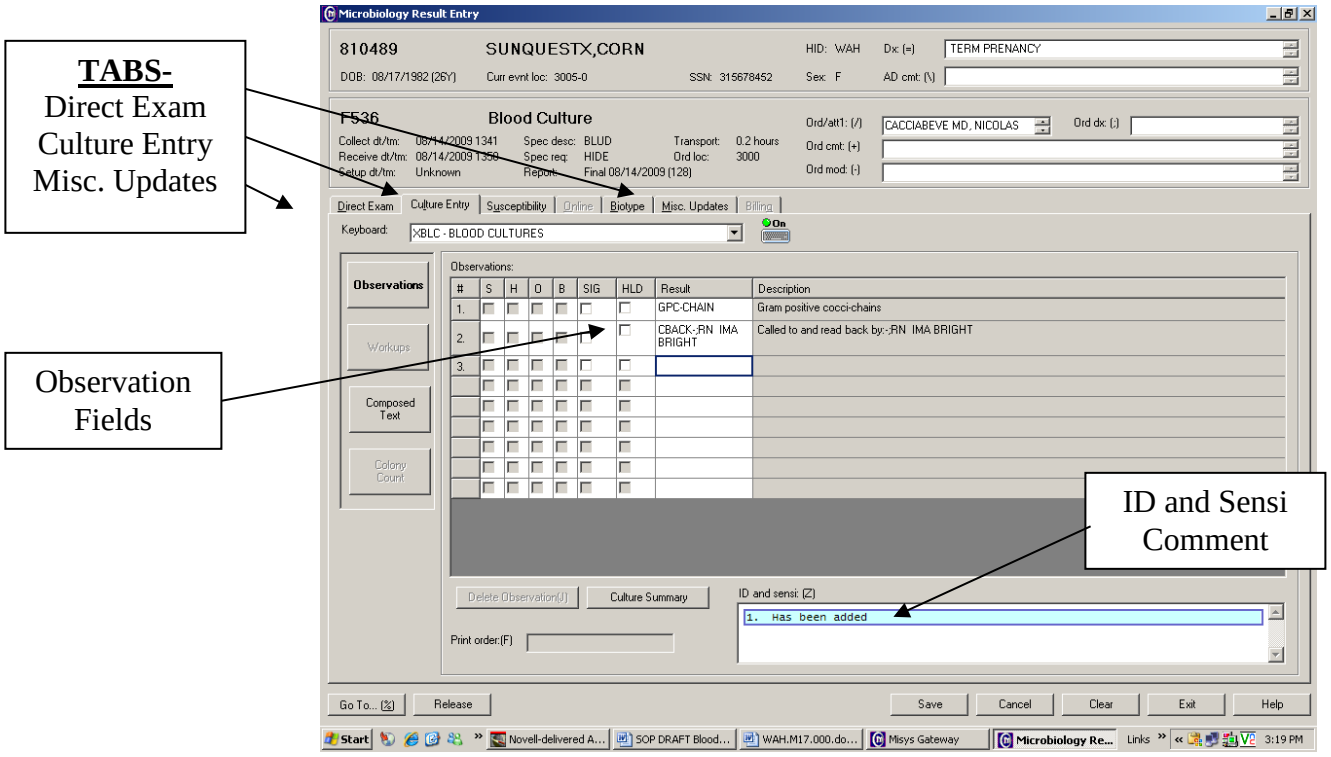
b. The accession data will appear in the lower part of the screen in the "Accession/Battery List". If the highlighted accession is the correct one, press **enter,** or click on it, or press **ALT + S.**
c. System will display the result entry screen. The screen opens automatically to the "Culture Entry" tab.
d. Click on the **Misc Updates** tab.
e. Double click on the **SDES** result and change BL to **BLUD**
f. Press tab three times to get out of the field. A message will appear that reads :

g. Click **OK**.
h. Select **Save**
i. Select **Save** again to exit.
j. From the Gateway screen go to Order Entry
k. Change "Lookup by:" to Accession Number
l. In the "Value" field, enter the accession number of the XBLC
m. Click **Search**
n. Click **Select** or ALT+C



o. Click "**Reprint Labels**" or ALT+L
p. Click "**Select All**" or ALT+S
q. Click "**Print**" or ALT+P
r. Click "**OK**"
s. **Exit.** After saving this, XBLC will generate Micro work cards. These are used to label the plates and worksheet with the Sunquest barcode label and for two technologists to record their codes and gram stain results.
t. Label plates with barcode labels (do not cover media type) and write the type of bottle (AER, ANA, or PEDS), date plated, and tech code near the bottom edge of the plate where it will not be covered by labels. Use a pencil to label a slide with accession number, patient last name, type of bottle (aer, ana, ped) and date positive.

2. Enter the ID and Susceptibility Note
   a. From the "Microbiology Result Entry" screen, Click on the **Direct Exam** tab.
   b. Arrow up to Observation line 1. (It will be default resulted as HIDE)
   c. Press **H** which will expand to "Has been added."
   d. DO **NOT** enter your gram stain results at this time.
      Note: Blood culture gram stain results are entered under the "Culture Entry" tab.
   e. Tab down to an empty observation field.
   f. Select **Save** to exit **or** click the **Culture Entry** tab to continue with entering gram stain results.



3. Result the Gram Stain
   a. From the "Microbiology Result Entry" screen, click on the **Culture Entry** tab. **Note**: Only gram stains for Blood Cultures are resulted in this field, result all other gram stains under the "Direct Exam" tab.
   b. Enter the gram stain result. One observation per line (one organism or cell type + quantity). **Do <u>NOT</u> go to the <u>Direct Exam</u> tab to result the Gram Stain.**
   c. After you have noted all observations, tab down to an empty observation line and type the following:
      ;CBACK<tab> ;; (Nurse or Dr.'s first and last names) **on** (month, day, and time), **by** (tech code). This will expand to "Called to and read back by:"

   d. Notify the appropriate nurse or doctor and document the call.
   e. Press the "/" to finalize the culture. This will finalize the gram stain, and another order will be added to enter the ID and Susceptibility results.
   f. Click on **Save** or press **ALT+ S**.
   g. Write the gram stain result on each plate.

4. Notification
   a. Positive Blood Cultures must be called to a nurse or doctor 24 hours a day, 7days a week
   b. Inpatient results are called to the floor.
   c. Outpatient results are called to the doctor's office during office hours and to the physician on call after hours.
   d. ER patients who have been discharged are called to the ER charge nurse.
   e. The first positive report on all positive gram stains must be called to the nurse or physician **BY A TECHNOLOGIST**.

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ALL POSITIVE GERMANTOWN EMERGENCY CENTER GRAM STAINS MUST BE CALLED TO THE CHARGE NURSE AT THE SGAH EMERGENCY DEPT TO ENSURE TIMELY FOLLOW UP.

Positive gram stains and cultures for both GEC and SGAH ER patients, not admitted, are called and faxed to the SGAH ER charge nurse.

5. Order the ID and Susceptibility
   a. The identification and susceptibility test code **MUST BE ORDERED ON THE ORIGINAL BLOOD CULTURE ACCESSION NUMBER.**
   b. From the Gateway screen, open SmarTerm, and log in.
   c. Enter function **REI** and enter the accession number for the positive bottle.
   d. At the prompt TEST-2: Add test code **XIDS** for a positive aerobic bottle or pediatric bottle and /or test code **XIDSN** for a positive anaerobic bottle.
   e. ACCEPT (A), MODIFY (M), OR REJECT (R)? enter **A**

Note: THIS IS THE *ONLY* CIRCUMSTANCE WHERE ADDING ADDITIONAL MICROBIOLOGY ORDERS TO THE SAME ACCESSION NUMBER IS ALLOWED.

C. Positive Gram Stain: Second Positive Bottle of a Set

1. If the gram stain result is the same as the first bottle **in the set,** there is no need to call the unit. Enter the gram stain result and tech ID on the worksheet only.
2. If the Gram stain result from the second bottle of a set is the same as the result from the first bottle **of the set,** a second read by another technologist is not required. Document the Gram stain result and tech ID on the worksheet and document the result and that the previous bottle was positive with same result.
3. Order the ID and Susceptibility on the same accession number using Test code **XIDS** for aerobic or pediatric bottles or **XIDSN** for anaerobic bottles. Refer to steps in B.5 above.
4. Label plates with barcode labels (do not cover media type) and write the type of bottle (AER, ANA, or PEDS), date plated, and tech code near the bottom edge of the plate where it will not be covered by labels. Follow procedure for sending plates.
5. If the Gram stain is different from the bottle previously reported, result the gram stain in Sunquest. Use code ADD (Addendum report) and free text “gram stain of additional bottle in set”. CALL all Addended results and document. Refer to steps in B.4 above.

D. Positive Gram Stain: Second Set from a Patient Drawn on Same Day

1. If the Gram stain result from a **second set** is the same as the result from the first set **of blood cultures from the same patient drawn on the same day,** there is no need to call the unit a second time.
2. If the Gram stain result from a subsequent set is the same as the result from a **previous set on the same patient,** a second read by another technologist is not required. Document the Gram stain result and tech ID on the worksheet and document that the previous set was positive with

the same Gram stain result and record the accession number of the previous set. Result the Gram stain in Sunquest. Refer to steps in B.4 above.

3. If the Gram stain result is different than reported on the previous set, follow the procedure in **B. Positive Gram Stain: First Positive Bottle of a Set.**
4. Order the ID and Susceptibility using Test code **XIDS** for aerobic or pediatric bottles or **XIDSN** for anaerobic bottles. Refer to steps in B.5 above.

E. Prepare Plates for Sendout

1. One set of blood culture plates per biohazard bag.
2. **Be sure to file the worksheet (which should include the gram stain results and tech codes of the two techs who read the slide) and the gram stain slide in established area.**
3. Plates are to be placed in the incubator until courier arrives for pickup.
4. Positive blood culture bottles are to be maintained at room temperature at the site reading the gram stain until the organism and sensitivity have been finalized by the reference site.

F. ROB

Print the Batch list and Packing list. Refer to the procedure ROB: Creating Batch for Microbiology Sendouts for details.

G. FES

FES must be performed for each order for **XIDS and XIDSN.** Refer to the procedure FES, Processing Microbiology Orders for details.

H. Overdue Log

Test codes **XIDS and XIDSN** are defined to worksheet **XBLC**. The number of days overdue is 6 days on this worksheet.

11. EXPECTED VALUES

11.1 Reference Ranges

No growth.

11.2 Critical Values

All positive blood cultures are critical values. The technologist reading the gram stain is responsible to make the first call to the unit/physician following the Laboratory Critical Value policy.

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

The detection of microorganisms in a patient's blood has diagnostic and prognostic importance. Bacteria multiply at a rate that exceeds the capacity of the reticuloendothelial system to remove microorganisms, bacteremia results. Bacteria usually enter the blood from extravascular sites via lymphatic vessels. Direct entry of bacteria into the bloodstream occurs as well with intravascular infections, such as infective endocarditis, infected arterio-venous fistulas, mycotic aneurysms, suppurative phlebitis, infected IV catheters, and infected indwelling arterial catheters. The clinical pattern of bacteremia can be transient, intermittent, or continuous, and bacterial sepsis constitutes one of the most serious infectious diseases. The expeditious detection and identification of blood-borne bacterial pathogens is one of the most important functions of the diagnostic microbiology laboratory.

13. PROCEDURE NOTES

- **FDA Status:** Approved/Cleared
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

- Antimicrobial therapy initiated prior to the collection of specimens may result in a false negative culture.
- Media specific for the recovery of fungus and mycobacteria are recommended. Refer to separate SOPs for Blood Culture, Fungus and Blood Culture, Mycobacteria.

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Safety Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Appropriate Personal Protective Equipment (PPE) must be worn at all times when handling blood culture specimens. Lab coat and gloves must be worn when loading or unloading the BACTEC™ instrument. The use of face shields for handling inoculated blood culture bottles is optional.
- Blood culture bottles should be handled with care at all times. The bottle necks are susceptible to breakage if they are struck against another object. Take extra care when

loading or unloading bottles from the BACTEC™ instrument since you will be grasping the neck of the bottles to perform these steps.

- Always use a subculture device to perform subculture and slide preparation. **Never use a standard syringe with needle attached.**
- For disposal place bottle into biohazard sharps container or suitable impermeable biohazard container.

Report all accidents and injuries to your supervisor or Safety Officer.

16. RELATED DOCUMENTS

Critical Values, Laboratory policy
Gram Stain, Microbiology procedure
Blood Culture Protocol, Phlebotomy procedure
Current package inserts for BD BACTEC ™ Plus Media
BACTEC Maintenance Log
ROB: Creating Batch for Microbiology Sendouts, Specimen Processing procedure
FES, Processing Microbiology Orders, Specimen Processing procedure
Positive Blood Culture Worksheet (AG.F211)
Blood Culture Gram Stain Referral / Consult Form (AG.F335)
Video Microscope (NetCam) M35

17. REFERENCES

- BD Package Inserts: PP-091J;02/2001, PP-088F;02/2001, PP-108E;01/2001.
- BACTEC ™ Fluorescent Series Users' Manual.
- Dunne, W.M., F.S. Nolte, and M.L. Wilson. 1997. Cumitech 1B, Blood Culture III. Coordinating ed., J. Hindler. American Society for Microbiology. Washington D.C.
- Isenberg, H.D., Editor-in-Chief. 2004. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D.C.
- Miller, J.M., H.T. Holmes, and K. Krisher, General Principles of Specimen Collection and Handling. In Murray, P.R. *et al.* Manual of Clinical Microbiology 8th ed., p. 59-60. American Society of Microbiology Press Washington DC.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP M023.003		
000	10/8/10	8.2	Delete visual inspection step	R. Master	R. Master
		11.2	Title change to local terminology	L. Barrett	R. Master /R. Strother
		16	Add current package inserts	L. Barrett	R. Master
		8.4	Edited removing negative bottles	R. Master	R. Master
001	10/25/11	Addendum A	BACTEC Maintenance Log moved to Related Documents	R. Master	R. Master

001	10/25/11	8.3.C	Added Safety Subculture Unit	R. Master	R. Master
		8.3.C	Added labeling of plates	R. Master	R. Master
		8.3.D	Added steps for smear negative bottles	R. Master	R. Master
		19	Update guide to new device	L. Barrett	R. Master
002	9/25/15	8.3 C.8	Add using loop to spread smear	R. Master	R. Master
		8.3 C.12	Add Previ stainer	R. Master	R. Master
		8.3 C.13	Delete reference to retired SOP	R. Master	R. Master
		8.3 D.1	Add to scan the patient bar code first	R. Master	R. Master
		8.3 D.4	Add completion of worksheet	R. Master	R. Master
		8.3.D.5	Edit to file slides in slide box	R. Master	R. Master
		8.3.D.6	Edited times for aerobic and anaerobic plates	R. Master	R. Master
		8.3 D.8	Deleted reference to retired SOP	R. Master	R. Master
		8.3.D.9	Added instructions for NOS bottle detected a second time	R. Master	R. Master
		10.6	Added M05 SOP to consolidate	R. Master	R. Master
		16	Added ROB, FES, Video Microscope & Referral form	R. Master	R. Master
		19	Added keyboard and flow chart	R. Master	R. Master
		Footer	version # leading zero's dropped due to new EDCS in use as of 10/7/13	L. Barrett	R. Master

19. ADDENDA

A. ITL Safety SubCulture Unit Quick Guide
B. Microbiology Blood Culture Keyboard
C. Positive Blood Culture Work Up Flow Chart

Safety SubCulture Unit — Quick Guide

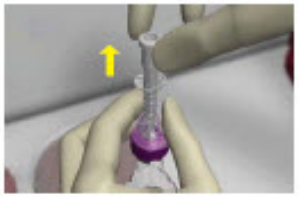
Note: This document is a product use reference guide. Please consult the package insert for the complete Instructions for Use.

Insert SCU

A) Remove SCU from package and position tip at center of disinfected bottle stopper.

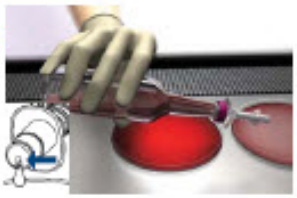
B) Hold bottle with one hand. With other hand, press down on SCU flanges to pierce bottle top.

Note: The SCU will not sit flush with the stopper.

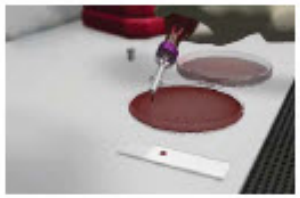


C) Hold SCU in place with one hand and using other hand, pull up on white filter cap to remove.

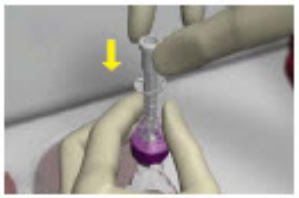
Drop Method



A) Position SCU sampling channel close to media plate or slide.

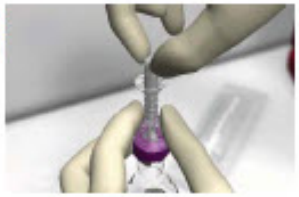


B) Tilt bottle to dispense drop.



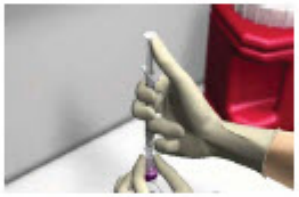
C) Replace white filter cap when finished dispensing drops.

Syringe Method



A) Aseptically remove the sampling channel. For example, place an alcohol wipe over insert and pull up with finger tips.

B) Insert male end of syringe into female luer of SCU. Invert bottle and syringe and withdraw required volume into syringe.



C) Upright bottle and syringe. Pull up on syringe plunger to clear any remaining culture in the SCU.

D) Replace white filter cap as described above in the Drop Method. Dispense sample from syringe.

Remove and Discard SCU

A) Hold bottle with one hand and with other hand twist and pull up on SCU to remove.

Discard into appropriate bio-hazard container.

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Sharps Safety & Best Practice

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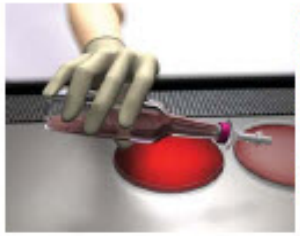
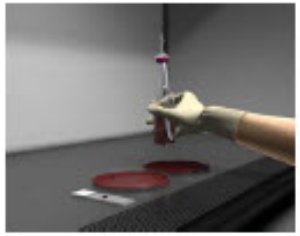
Safety SubCulture Unit — Tips & Tricks

General Guidelines

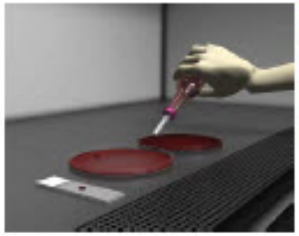


Tilt bottle to smaller angle (approx. 30-45 degrees) above horizontal for better control of drop size and speed.

Note: Gaseous samples tend to flow more quickly.

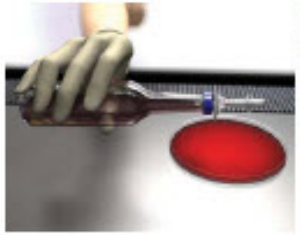


Prepare to upright bottle quickly to cut off the drop size and rate.

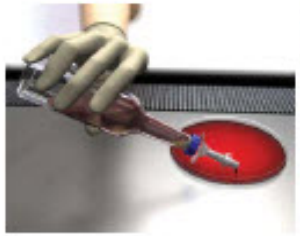


When dispensing to a series of slides or other media, dispense drops sequentially without uprighting the bottle between drops.

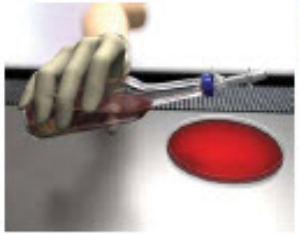
Resin Media



To reduce potential clogging, turn the bottle horizontal allowing the resin to settle along the side of the bottle prior to dispensing drops.

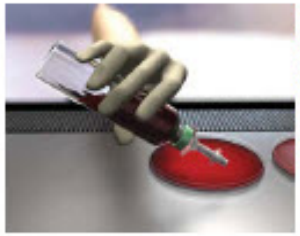


Increase the angle of the bottle to dispense drops.



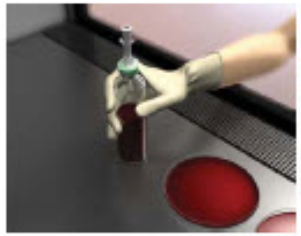
If resin obstructs the SCU tip inside the bottle, tilt the wide end of the bottle downward to clear the resin from the tip. Allow the resin to settle along the side of the bottle prior to dispensing additional drops.

Charcoal Media



To reduce potential clogging, tilt the bottle approx. 30-45 degrees above horizontal. Do not invert the bottle, as this may increase clogging.

Clogging may be cleared by gently pressing the base of the SCU against the septum while the bottle is at a slight angle.



Clogging may also be cleared by replacing the SCU filter cap and gently tapping the base of the bottle on a counter.

An alternative is to use the syringe method.

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Addendum B

BLOOD CULTURE KEYBOARD
Result / Modifier Keys

ESC	F1 EXIT MAILBOX	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11 EXIT	F12

! 1 **RARE**	@ 2 **FEW**	# 3 **MOD**	$ 4 **MANY**	% 5 **NOCO**	^ 6 **NTY**	& 7 **HYPH**	* 8 **YPSU**	(9 **GT**	) 0 **GNCB**	-	+	Back Space ←

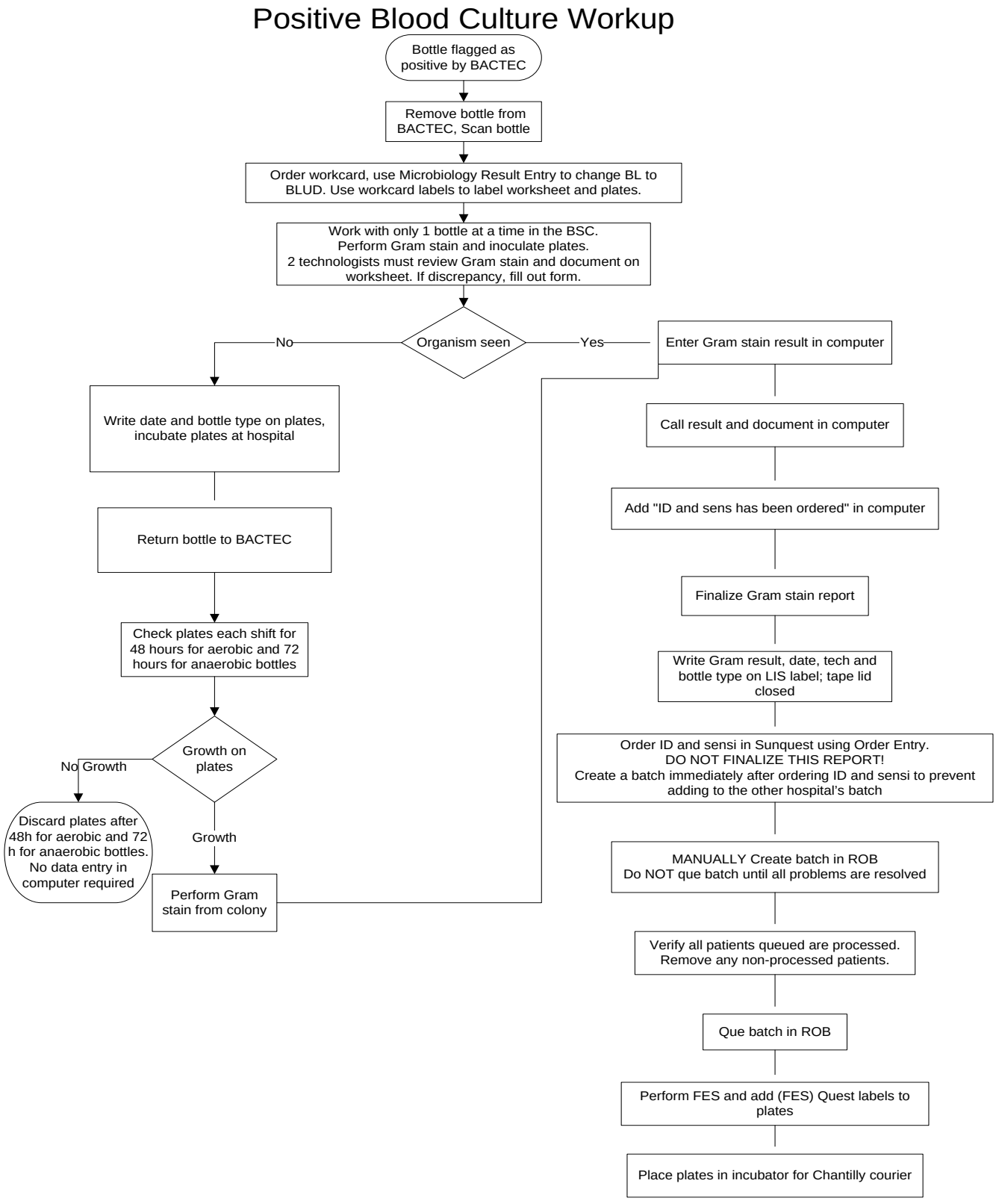
Q **CLUE**	W **WBCS**	E **EPIT**	R **RBCP**	T **TRIC**	Y **YST**	U **GPR**	I **GNR**	O **NOS**	P **POSIT**	{ / [**UNIN**	}] **INVAL**	\

A **PCPR**	S **GPRD**	D **GNDC**	F **BGPR**	G **GPCN**	H **GPC**	J **CHAIN**	K **CLUST**	L **PAR**	; **OTHR**	‘ ←	ENTER ↵	

Z **INTRA**	X **EXTRA**	C **GNC**	V **GVCB**	B **GVR**	N **ng** / n **HIDE**	M **GNCB**	,	.	/ **fnl** / ? **PREVR**

Form revised 10/31/02

Addendum C



Form revised 10/31/02

Quest Diagnostics™

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☐ Washington Adventist Hospital

Blood Culture Gram Stain Referral / Consult Form

* This form must be completed for all Blood Culture Gram stains not reported and held for the next shift to read and report.

Misys Accession #: _____

Aerobic Bottle / Anaerobic Bottle [circle the correct bottle(s)]

Patient Name: _____

Date: _____

1st Technologist Name: _____ Tech code: _____

Gram stain impression:

2nd Technologist Name: _____ Tech code: _____

Gram stain impression:

Agreement: Y / N

Reason for Referral:

Result Completed Date: _____ Technologist: _____