



HEALTH HOLDING

HAFER ALBATIN HEALTH  
CLUSTER  
MATERNITY AND  
CHILDREN HOSPITAL

<b>Department:</b>	Laboratory and Blood Bank (Microbiology)		
<b>Document:</b>	Internal Policy and Procedure		
<b>Title:</b>	Automated Blood Culture: Bact/ALERT 3D and BD BACTEC 9120		
<b>Applies To:</b>	All Laboratory Staff		
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## 1. PURPOSE:

- 1.1 To establish system and set responsibilities for work.
- 1.2 To elucidate the accurate operating procedure for Bact/ALERT 3D and BD BACTEC 9120 machines.

## 2. DEFINITONS:

- 2.1 N/A

## 3. POLICY:

- 3.1 The accurate operating procedure for Bact/ALERT 3D and BD BACTEC 9120

## 4. PROCEDURE:

### 4.1 General safety considerations:

- 4.1.1 Pathogenic microorganisms, including Hepatitis viruses and Human Immunodeficiency Virus, may be present in specimens. "Standard and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids."
  - 4.1.1.1 Wear gloves while handling inoculated vials.
  - 4.1.1.2 Perform all blood culture processing in a biological safety cabinet.
  - 4.1.1.3 Properly dispose of all contaminated materials. (Place syringes, needles, and other sharp contaminated materials in a puncture proof container).

### 4.2 Processing new blood cultures:

- 4.2.1 To load instrument with Blood culture bottles, follow Steps described below:
  - 4.2.1.1 Using Bactec/ Alert 3D:
    - 4.2.1.1.1 Tap the vial load icon on the status display
    - 4.2.1.1.2 The drawer with available stations will show green light, open it.
    - 4.2.1.1.3 The barcode scanner turns on.
    - 4.2.1.1.4 Scan a vial sequence barcode label.
    - 4.2.1.1.5 Place the vial into an available station (solid green indicator). Repeat the process until all vials are loaded.
    - 4.2.1.1.6 Tap the "check button" on the status screen then close the drawer.
  - 4.2.1.2 Using BD Bactec 9120:
    - 4.2.1.2.1 Open the door.
    - 4.2.1.2.2 Use scanner on the right door to scan The **Vial Entry** Barcode On the left door.
    - 4.2.1.2.3 All available stations will enlighten.
    - 4.2.1.2.4 Scan the sequence barcode on each vial & place it in the specified enlightened station.
    - 4.2.1.2.5 Repeat the process until all vials are entered then close the door.
    - 4.2.1.2.6 On the status screen, loaded Stations colour turns into BLUE.
- 4.2.2 Avoid opening the drawer/ door unnecessarily. Drawers should not remain open longer than **10 minutes**. Make sure all vials are fully inserted in the stations before closing the drawer/

door.

#### 4.2.3 Anonymous Vial Entry:

- 4.2.3.1 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.
- 4.2.3.2 Anonymous vials are recognized by the instrument when they are placed in stations. They follow the default protocol of 5 days. Anonymous vials are evaluated with general positivity criteria.
- 4.2.3.3 Anonymous vials are displayed as "Anonymous!"
- 4.2.3.4 If the instrument notifies anonymous vial, you can remove & reload again (follow the steps of new vial entry).

#### 4.3 Positive, Negative and Anonymous Vials:

##### 4.3.1 Notification of positive, negative & anonymous vials:

- 4.3.1.1 **Bactec/ Alert 3D** display notifies you of new positive, negative & anonymous vials:
  - 4.3.1.1.1 On the Status display, the "Unload positives" button is active (blue), the display background turns into yellow and the number of positive vials in the drawer is shown.
  - 4.3.1.1.2 On the Status display, the "Unload negatives" button is active and the number of negative vials in the drawer is shown (the same for anonymous vials).
- 4.3.1.2 **BD Bactec 9120** notifies positive, negative & anonymous blood culture vials as follows:
  - 4.3.1.2.1 On the status display, positive vial station(s) turns into FLASHING RED colour in addition to flashing red colour in lower part of the monitor.
  - 4.3.1.2.2 On the status display, negative vial station (s) turns into GREEN.

##### 4.3.2 Removing positive vials:

- 4.3.2.1 If **Bactec/ Alert 3D** display notifies you of positive blood cultures vial:
  - 4.3.2.1.1 On the Status display, press the "**Unload positives**" button.
  - 4.3.2.1.2 The drawer that has positive vials will illuminate, open it.
  - 4.3.2.1.3 All positive vial station(s) illuminate GREEN, remove all.
  - 4.3.2.1.4 Close the door & press the "check button" on the status screen.
  - 4.3.2.1.5 The display is updated dynamically as vials are removed.
- 4.3.2.2 If **BD Bactec 9120** display notifies you of positive blood culture vial(s):
  - 4.3.2.2.1 Open The door, scan "remove positive" barcode on the left door.
  - 4.3.2.2.2 The positive vial(s) will illuminate, remove one by one.
  - 4.3.2.2.3 **After removing each vial, scan the barcode sequence on it.**
  - 4.3.2.2.4 Remove all positive vials then close the door.
  - 4.3.2.2.5 The display is updated dynamically as vials are removed.

##### 4.3.3 Removing negative vials (after 5 days incubation):

- 4.3.3.1 If **Bactec/ Alert 3D** display notifies you of negative blood cultures vial(s):
  - 4.3.3.1.1 On the Status display, press the "Unload negatives" button
  - 4.3.3.1.2 The drawer that has negative vials will illuminate, open it.
  - 4.3.3.1.3 All negative vial station(s) illuminate GREEN, remove all
  - 4.3.3.1.4 Close the door & press the "check button" on the status screen.
  - 4.3.3.1.5 the display is updated dynamically as vials are removed.
- 4.3.3.2 If **BD Bactec 9120** display notifies you of negative blood culture vial(s):
  - 4.3.3.2.1 Open The door, scan "remove negative" barcode on the left door.
  - 4.3.3.2.2 The negative vial(s) will illuminate, remove all.
  - 4.3.3.2.3 Close the door.
  - 4.3.3.2.4 The display is updated dynamically as vials are removed.

##### 4.3.4 Processing Positive Vials:

- 4.3.4.1 Remove the vial from the instrument and place in a biological safety cabinet.
- 4.3.4.2 Invert the vial to mix the contents & wipe its top rubber with 70% alcohol.
- 4.3.4.3 Using syringe, aspirate about 1ml of the vial content for making wet film, gram smear preparation & media inoculation on (blood, MacConkey's & Chocolate agar).

- 4.3.4.4 Gram stain the smear, read it, report preliminary results
- 4.3.4.5 Subculture vials according to the Gram stain results.
- 4.3.4.6 Perform identification and susceptibility.
- 4.3.5 Changing the Maximum Test Time — Individual Bottles
  - 4.3.5.1 From the Load screen, press the Change Maximum Test Time button after scanning the bottle barcode.
  - 4.3.5.2 The Change Maximum Test Time screen overlays and disables the Load screen.
  - 4.3.5.3 Verify the bottle ID matches that of the bottle for which you wish to change the maximum test time.
  - 4.3.5.4 Adjust the maximum test time in days using the Max Test Time scroll buttons.
  - 4.3.5.5 Press the Check button to accept the changes, or the Cancel button to retain the original setting.
  - 4.3.5.6 The system returns to the Load Mode screen.
  - 4.3.5.7 **Note:** Changing the maximum test time of an individual bottle during loading does not affect any other bottles of the same type.

#### 4.4 **Quality control:**

- 4.4.1 Blood culture vials:
  - 4.4.1.1 DO NOT USE culture vials past their expiration date.
  - 4.4.1.2 DO NOT USE culture vials that exhibit any cracks or defects; discard the vial in the appropriate manner. Each case of media has a Quality Control certificate indicating the organisms tested and the acceptability of those tests.
  - 4.4.1.3 A positive and negative vial may be used to test the performance of the media. The positive vial should be inoculated with 1.0 mL of a 0.5 McFarland Standard of either **Escherichia coli** or **Staphylococcus aureus** prepared from a fresh 18 – 24 h culture.
  - 4.4.1.4 This vial and an un-inoculated vial should be logged into the instrument and tested.
  - 4.4.1.5 The inoculated vial should be detected as positive by the instrument within 72 hours.
  - 4.4.1.6 The negative control vials should remain negative throughout the entire testing protocol.

#### 4.5 **Instrument maintenance:**

Each day several simple maintenance procedures should be performed. The best time to perform maintenance is first thing in the morning, but it may be done at any time you find convenient. The following procedures should be performed.

- 4.5.1 tap the "status" button to illuminate the system status indicators on the mullions. Both sides of all the indicators (amber, red, and green) should illuminate. If any indicator does not light, contact BD representative for service.
- 4.5.2 Check the temperature on the temperature vial(s).
- 4.5.3 Information can be recorded on the Maintenance QC Report Forms.

### 5. **MATERIAL AND EQUIPMENT:**

#### 5.1 **Blood culture vials:**

- 5.1.1 For Bactec/ Alert 3D: See appendix 7.1
  - 5.1.1.1 Standard aerobic (SA) (with blue cap) & anaerobic (SN) (with violet cap) media.
  - 5.1.1.2 Fastidious Antimicrobial neutralization (FAN) media with activated charcoal; FA FAN for aerobic (light green cap), FN FAN for anaerobic (orange cap)& PF paediatric FAN (yellow cap).
- 5.1.2 For BD Bactec 9120: See appendix 7.2

### 6. **RESPONSIBILITIES:**

- 6.1 Assigned technician/ technologist for microbiology lab.: processing of blood culture & release of negative results.
- 6.2 C. pathology specialist/ consultant: interpretation of results


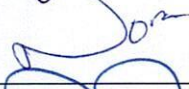


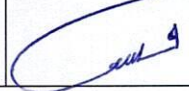
**7. APPENDICES:**

- 7.1 Blood culture vials of Bactec/ alert 3D
- 7.2 Blood culture vials of BD Bactec 9120
- 7.3 Calibration of an instrument cell (Bactec/ Alert 3D)

**8. REFERENCES:**

- 8.1 Automated blood culture instrument procedure manual of BD Bactec 9120 & Bactec Alert 3D



**9. APPROVALS:**

	Name	Title	Signature	Date
<b>Prepared by:</b>	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 05, 2025
<b>Reviewed by:</b>	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 08, 2025
<b>Reviewed by:</b>	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
<b>Reviewed by:</b>	Dr. Tamer Mohamed Naguib	Medical Director		January 13, 2025
<b>Approved by:</b>	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025

Appendix 7.1



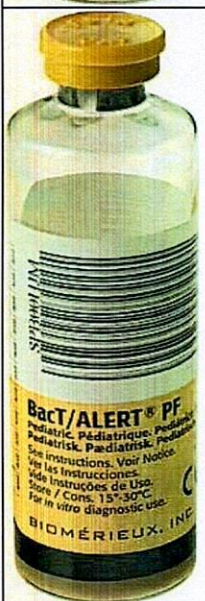
BLOOD CULTURE VIALS OF BACTEC/ ALERT 3D

I. Standard Media

Bottle Type	Media Formulation	Specimen Type	Specimen Volume
 <p>BACT/ALERT® SA Standard Aerobic Ref: 259789</p>	<p>40 mL supplemented tryptic soy broth (TSB)</p>	<p>Blood or normally sterile body fluid (SBF)</p>	<p>Up to 10 mL</p>
 <p>BACT/ALERT® SN Standard Anaerobic Ref: 259790</p>	<p>40 mL TSB</p>	<p>Blood or SBF</p>	<p>Up to 10 mL</p>

**II. Fastidious Antimicrobial Neutralization Media (FAN®):**

**FAN MEDIA WITH ACTIVATED CHARCOAL TO NEUTRALIZE ANTIMICROBIALS**

Bottle Type	Media Formulation	Specimen Type	Specimen Volume
 <p>A clear glass vial with a green cap and a green label. The label reads: Bact/ALERT® FA Aerobic, Aérobie, Aeróbico, Anaerobio, Anaeróbica, Anaeróbico, Anaerobio. See instructions. Voir Notice. Ver las Instrucciones. Use Instruções de Uso. Store / Cons. 15°-30°C. For in vitro diagnostic use. BIOMÉRIEUX, INC.</p>	<p>BACT/ALERT® FA FAN® Aerobic Ref: 259791</p>	<p>30 mL peptone-enriched TSB, supplemented with Brain Heart Infusion (BHI) solids and activated charcoal</p>	<p>Blood or normally sterile body fluid (SBF)</p> <p>Up to 10 mL</p>
 <p>A clear glass vial with an orange cap and an orange label. The label reads: Bact/ALERT® FN Anaerobic, Anaeróbica, Anaeróbico, Anaerobio, Anaeróbica, Anaeróbico, Anaerobio. See instructions. Voir Notice. Ver las Instrucciones. Use Instruções de Uso. Store / Cons. 15°-30°C. For in vitro diagnostic use. BIOMÉRIEUX, INC.</p>	<p>BACT/ALERT® FN FAN® Anaerobic Ref: 259793</p>	<p>40 mL peptone-enriched TSB, supplemented with BHI solids and activated charcoal</p>	<p>Blood or SBF</p> <p>Up to 10 mL</p>
 <p>A clear glass vial with a yellow cap and a yellow label. The label reads: Bact/ALERT® PF Pediatric, Pédiatrique, Pediátrico, Pediatrik, Pediátrik, Pediátrik, Pediátrik. See instructions. Voir Notice. Ver las Instrucciones. Use Instruções de Uso. Store / Cons. 15°-30°C. For in vitro diagnostic use. BIOMÉRIEUX, INC.</p>	<p>BACT/ALERT® PF Pediatric FAN® Ref: 259794</p>	<p>20 mL peptone-enriched TSB, supplemented with BHI solids and activated charcoal</p>	<p>Blood</p> <p>Up to 4 mL</p>

**BD BACTEC 9120 Blood Culture Media**



**BACTEC PEDS PLUS (Pink Cap) ----- for pediatric age group**

**BACTEC Standard Aerobic/F (Blue Cap) ----- for adult age group (aerobic)**

**BACTEC Standard Anaerobic/F (Yellow Cap) ----- for adult age group (anaerobic)**

**BACTEC PLUS + Aerobic (Gray Cap) ----- for adult age group (aerobic)**

**BACTEC PLUS + Anaerobic (Orange Cap) ----- for adult age group (anaerobic)**

## CALIBRATING AN INSTRUMENT CELL

### I. The following procedures describe how to:

1. Locate a cell which failed calibration
2. View a cell's readings
3. Calibrate a cell
4. **Note:** It is not necessary to perform routine cell calibration. If a cell fails its automatic internal diagnostic check, **Instrument Fault Code 60** appears in the Instrument icon on the Main screen.
5. **CAUTION:** A cell which has failed the automatic internal diagnostic check no longer records bottle readings and will not be indicated as available when loading bottles. The cell must be calibrated or disabled.
6. **CAUTION:** Do not invoke the Calibrate Cell screen unless the cell to be calibrated is empty.

### II. Locating a Cell Which Failed Calibration:

1. View the Main screen to locate the Instrument Fault Code 60 in the Instrument icon.
2. Locate the cell with the fault (see Viewing Faults in Chapter 3 for Clinical Use)
3. Note: If the cell cannot be calibrated immediately, disable the cell (see Enabling and Disabling Modules, Drawers, Racks, and Cells on page 8-18).
4. **CAUTION:** To prevent anonymous loading into the disabled cell, place an orange cell plug into the cell.

### III. Viewing a Cell's Readings and/or Calibrating a Cell:

1. If calibrating, print out the Cell Calibration Report (see Viewing and Printing Calibration Data in Chapter 7).
2. If the cell to be calibrated contains a bottle, then relocate it (see Relocating Bottles on page 8-21).
3. Access the Setup screen and enter a valid password (see Accessing the Setup Screen Function Buttons in Chapter 3 for Clinical Use, or in Chapter 4 for INDUSTRY Use).
4. From the Setup screen, press the Calibrate Cell button ( ).
5. The Calibrate Cell screen overlays and disables the Setup screen.
6. Select a particular cell to be calibrated:
  - Choose the appropriate Incubation or Combination Module (1–6) with the Incubation Module scroll button.
  - Choose the appropriate drawer (A–D) with the Drawer scroll button.
  - Choose the appropriate cell (1–60) with the Cell scroll buttons. The first screen displays an X at the base of the staircase icon. This prompts the user to verify the cell is empty and the drawer is closed.
  - **Note:** The Module, Drawer, and the Cell scroll buttons will be grayed-out and inactive until calibration of the cell is complete.

### IV. To view a cell's reading:

1. The cell's readings can now be read below the Cell scroll buttons. The first set of numbers represents the **un-calibrated** reading and the number to the right of the arrow represents the calibrated readings.
2. **Note:** If an **incubation** module is not configured properly or is not responding, asterisks will replace the digits on the screen.

**V. If you would like to calibrate a cell, proceed to next step.**

1. Press the Check button. The cell light of the cell selected for calibration is now illuminated and #1 appears above the first step of the Calibration Staircase icon.
2. Insert Standard Number One into the selected cell without touching the ends of the calibration standard as calibration could be affected. (A single ring around the end of the reflectance standard identifies Standard #1).
3. Press the Check button. A #2 appears above the second step of the Calibration Staircase icon.
4. Insert Standard Number Two into the selected cell. (Two rings around the end of the reflectance standard identifies Standard #2).
5. Press the Check button. A #3 appears above the third step of the Calibration Staircase icon.
6. Insert Standard Number Three into the selected cell. (Three rings around the end of the reflectance standard identifies Standard #3).
7. Press the Check button. A #4 appears above the fourth step of the Calibration Staircase icon.
8. Insert Standard Number Four into the selected cell. (Four rings around the end of the reflectance standard identifies Standard #4).
9. Press the Check button.
10. If a check mark appears at the top of the Calibration Staircase icon, then the calibration of the cell was successful. Remove Standard Number Four and press the Check button to save the new calibration values.
11. If Instrument Fault Code 60 appears at the top of the Calibration Staircase icon, then the calibration of the cell is unsuccessful. Press the Cancel button and follow Step 4 through Step 14 to recalibrate the cell.
  - Note: The cell is not automatically enabled after calibration is completed successfully. If the cell was previously disabled, it must be enabled (see Enabling and Disabling Modules, Drawers, Racks, and Cells on page 8-18).
12. Press the Previous Screen button to return to the Setup screen. Note: The calibration process can be canceled and any new calibration values discarded by pressing the Cancel button at any step during the calibration process.
13. Print out the Cell Calibration Report (see Viewing and Printing Calibration Data).