KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 22 Augusr 2006	SOP-Number MIC.001
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Title: OPERATION OF BacT/ALERT™ 3D MICROBIAL DETECTION SYSTEM

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By	Date

Title: OPERATION OF BacT/ALERT™ 3D MICROBIAL DETECTION SYSTEM

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Title: OPERATION OF BacT/ALERT™ 3D MICROBIAL DETECTION SYSTEM

PURPOSE

Instructions for operation of the bioMerieux BacT/ALERT 3D instrument.

SCOPE

This Standard Operating Procedure applies to all personnel in the KCMC Biotechnology Laboratory, microbiology section who process and evaluate blood cultures in the BacT/ALERT instrument.

PRINCIPLE OF OPERATION

As microorganisms metabolize the substrates in the media, carbon dioxide is produced causing a change in the color of the sensor on the bottom of the vials from dark grey to light yellow. A Light Emitting Diode (LED) projects light into the sensor and the light reflected is measured by a photodetector. As more CO_2 is generated, more light is reflected and this is compared to the initial sensor reading. If there is a sustained acceleration in the rate of CO_2 production or there is a high initial CO_2 content, the sample is flagged as positive. In the case of mycobacterial growth which is slower, vials showing either an abrupt or slow sustained rise in CO_2 production are flagged as positive. If the CO_2 level does not change significantly after a specified number of days the sample is flagged as negative. Bottles flagged as positive are unloaded from the instrument and aliquots removed for microscopic examination and culture using conventional methods.

STANDARD PRECAUTIONS

Observe standard precautions and wear gloves when handling patient specimens to prevent exposure to bloodborne pathogens. Consult KCMC Standard Operating Procedure SAF.002 BIOHAZARD SAFETY for complete information.

EQUIPMENT/SUPPLIES

BacT/ALERT 3D Microbial Detection Instrument

Inoculated culture bottles (SA, PF, MB, MP – refer to chart below for descriptions)

Culture Bottles

BacT/ALERT BOTTLES/RECOVERY	MEDIA VOLUME	MEDIUM	ATMOSPHERE
SA –aerobic bacteria	40 ml	Pancreatic digest of casein, papaic digest of soybean meal, peptones and CHO substrates.	CO_2 in oxygen
PF (Pediatric) – aerobic and facultative anaerobic bacteria and yeast	20 ml	16 ml of supplemented soybean-casein digest and brain heart infusion solids and 4 ml of an 8.5% charcoal suspension.	CO_2 in oxygen and nitrogen
MB – mycobacteria from blood specimens	29 ml	Supplemented Middlebrook 7H9 broth with SPS anticoagulant. Requires addition of MB/BacT Enrichment fluid containing a lytic agent.	CO_2 in oxygen and nitrogen
MP – mycobacteria from non-blood specimens	10 ml	Supplemented Middlebrook 7H9 broth. Requires addition of MB/BacT Antibiotic Supplement to inhibit bacterial growth in contaminated specimens and MB/BacT Reconstitution Fluid for sterile specimens.	CO_2 in oxygen and nitrogen

INCUBATION MODULES

The BacT/ALERT instrument has 2 Incubation Modules. Each module contains 4 drawers (labeled A,B,C,D). The upper module (MB) is for incubating mycobacterial culture vials, the lower (BA) is for bacterial cultures. Each drawer contains 3 racks with 20 cells for a total of 60 cells per drawer (numbered 1-60). Each cell holds and monitors one bottle.

MODULE TEMPERATURE/INCUBATION TIME SETTINGS

MODULE	TEMPERATURE	INCUBATION
MB	37^{o} C	42 Days
BA	35^{o} C	5 Days

DRAWER INDICATOR LIGHTS

Indicator lights are located on the outside of each drawer:

Yellow – illuminates when the drawer is open and flashes if drawer is left open too long or there is an error associated with the drawer.

Green – illuminates when user has selected an operation (e.g. loading/unloading bottles), flashes when drawer is left open too long.

MAIN SCREEN (2.3)*
*Reference page in Operator Manual

The BacT/ALERT system is monitored from the main screen and all functions are accessed from the screen. Information about the number of positive bottles detected and negative bottles to be unloaded is displayed and various functions such as loading and unloading bottles can be performed by touching the screen icons. The background color will change if a positive bottle is detected or an instrument fault occurs.

Background colors:

Yellow – indicates that the instrument has detected a positive bottle.
Red - indicates that an instrument fault has occurred and the fault number code will be displayed (consult chart of fault codes located in the keyboard). This can be overridden by touching the screen.

PROCEDURES

LOADING BOTTLES (2.9)

1. Log in bottles according to instructions in MIC.002 BacT/ALERT and MycoFLYTIC Blood Cultures for Bacteria and Mycobacteria.
2. Check the bottle to make sure it is not cracked.
3. Check the sensor on the bottom of the vial. If the sensor is yellow, treat the bottle as a positive.
4. Weigh the bottle and record original and inoculated weights on data form.
5. Press the LOAD BOTTLES icon on the Main Screen to access the Load Mode screen.
6. Scan the bar code on the side of the bottle and verify that the correct bottle type is displayed on the screen.
7. Type in the study number using the keyboard.
8. Open appropriate module drawer (BC or MB) that displays a green light indicating available cells.
9. Insert the bottle, sensor end first, into any available cell.
10. Repeat steps 1-8 for each bottle to be loaded.
11. Close drawer completely.
12. Press the CHECK icon to exit the Load Mode screen (this screen will disappear after 2 minutes of non-activity.)

UNLOADING BOTTLES (2.12)

1. The background of the MAIN SCREEN will turn yellow to indicate presence of positive bottle(s).
2. Select the POSITIVE BOTTLE icon. Green lights will be lit on the drawers containing positive bottles. Note the drawers containing positive bottles.
3. Go to the SET UP SCREEN: select arrow on bottom right to access. Enter password (1234) and press the key icon to unlock the screen.
4. Select the EDIT CELL CONTENTS icon (bottom of 2nd row).
5. Use the scroll buttons on the bottom of screen to locate the drawer(s) containing positive bottles.
6. Cells containing positive bottles will be highlighted with a yellow circle.
7. Press the cell to access the bottle history for the “time to positive”.
8. The time to positive can be found directly above the Positive Bottle icon (a book and a clock). Record the number on the BLOOD CULTURE FORM in the TIME TO POSITIVE boxes. Time to positive is expressed in percentage of days.
9. Remove the bottle from the cell. The cell light will blink and beep to acknowledge removal of the bottle.
10. Select the red check mark on the pop-up screen.
11. Proceed with examination of bottles flagged positive. Refer to MIC.002 BacT/ALERT and MycoFLYTIC Blood Cultures for Bacteria and Mycobacteria.

UNLOADING NEGATIVE BOTTLES (2.12)

1. Press the NEGATIVE UNLOAD icon.
2. Green indicator lights will be illuminated on the drawers containing bottles of the selected type.
3. Open the drawer. Cell indicator lights will be illuminated next to cells containing bottles of the selected type.
4. Remove the bottle from the cell and wait for the cell light to blink slowly to acknowledge the removal of the bottle.
5. Close all drawers completely.
6. Press the CHECK icon.
7. Report negative findings on the BLOOD CULTURE FORM and issue a negative report.

VIEWING CELL STATUS (2.5)

A continuously updated status of bottles in each drawer can be viewed on the VIEW CELL STATUS screen. Locations of loaded and empty cells, positive, negative and negative to date bottles, disabled cells and cell faults can be identified. Refer to page 2.5 of the Operator Manual for a description of the cell displays.

1. Touch the appropriate Module (BC or MB) on the Instrument icon in the middle of the Main Screen to access the View Cell Status screen.
2. Select the drawer using the DRAWER SELECTION icon on the lower right of the screen.
3. Determine status of individual cells.

FALSE POSITIVE BOTTLES (2.13)

"False Positive" - a bottle that has been flagged as positive that does not reveal any organisms on microscopic examination.

1. Subculture to appropriate media based on bottle type (e.g. SA bottle to Blood, Chocolate agars). Refer to MIC.002 Refer to MIC.002 BacT/ALERT and MycoFLYTIC Blood Cultures for Bacteria and Mycobacteria procedure.
2. Scan the barcode and reload the bottle in the instrument.
3. The bottle status will revert to "negative to date" once a reading has been taken.
4. If the subculture of the bottle is positive then the bottle status must be edited to Positive using the Edit Test Result Screen (see next section).

EDITING BOTTLE STATUS -NEGATIVE TO POSITIVE (3.2-3.5)

1. On the Main screen press the NEXT SCREEN icon (arrow in right lower corner).
2. Enter the password (1-2-3-4) and press the key icon. This will unlock the padlock and allow access to the functions on the Setup Screen (2.22,23).
3. Press the SELECT BOTTLE TO EDIT/GRAPH icon (Book/bottle display) to access the Edit/Graph screen.
4. Scan the barcode to enter the Bottle ID or enter number manually (number is on bar code on vial).
5. If the bottle is still in the instrument enter the module, drawer and cell location of the using the appropriate scroll buttons to display the location.
6. Press the CHECK icon to access the Edit Bottle Detail Screen.

7. Press the EDIT TEST RESULT icon (hand with pointing finger on lower right of screen) to access the pop-up screen to edit result.
8. Touch the checkbox next to the Positive status to insert an "X".
9. Press the CHECK icon to accept the change.

NOTE: Bottle status can also be edited using the Edit Cell Contents Screen. Access the SETUP screen and press the EDIT CELL CONTENTS icon (book with cell). Select the module and drawer using the selection buttons and then touch the appropriate cell icon to access the Edit Bottle Detail screen

CHANGING MAXIMUM TEST TIME (3.6)

Both BA and MB modules have been preset to specific maximum incubation times (BA module - 5 days for bacterial cultures and MB module – 42 days for mycobacterial cultures). Should it be necessary to extend the incubation time (e.g. suspected slow growing organism) this can be done on the Edit Bottle Detail screen.

1. On the Main screen press the NEXT SCREEN icon (arrow in right lower corner).
2. Enter the password (1-2-3-4) and press the key icon. This will unlock the padlock and allow access to the functions.
3. Press the SELECT BOTTLE TO EDIT/GRAPH icon (Book with bottle display) to access the Edit/Graph screen.
4. Enter the module, drawer and cell location of the using the appropriate scroll buttons to display the location.
5. Press the CHECK icon to access the Edit Bottle Detail Screen.
6. Using the scroll arrows on the EDIT MAXIMUM TIME icon (bottle with clock on middle left of screen) change to the desired number of days for maximum incubation.

FAULTS AND TROUBLESHOOTING PROCEDURES

VIEWING FAULTS (2.4)

Instrument faults are reported using a numeric code within a diamond shape that is displayed on the Instrument icon on the Main Screen where the fault condition exists. The background color of the main screen also turns to red when there is a fault recognized.

1. If the fault code appears on the top half of the Combination Module refer to the Fault Code list for a description of the code and SYSTEM TROUBLESHOOTING section of the Operator Manual for probable causes and solutions.

2. If the fault code appears on an incubation module or bottom half of the Combination Module touch the incubation or Combination Module on the Instrument icon on the main screen that contains the fault code. The VIEW CELL STATUS screen will appear.
3. Determine of the fault is in a module, drawer, rack or cell and, based on the fault code determine if module, drawer, rack or cell needs to be disabled.
4. Document faults and action taken on the BacT/ALERT Faults Log.

DISABLING MODULE, DRAWER, RACK OR CELL (5.14)

Before disabling any part of the instrument, bottles incubating in the location to be disabled must be relocated.

1. Relocating bottles (5.15):
    a. To relocate bottles into another available cell, rack or drawer go to the Main Screen and press CTRL+10 to enter a special Bottles Relocation mode. The indicator lights in loaded cells will be continuously lit, unloaded cells will be unlit.
    b. Pull bottles out <u>one at a time</u> and reload <u>one at a time</u> into any available cell.
    c. When all bottles are reloaded press the CHECK icon on the Main Screen.
2. Access Setup Screen by selecting the arrow icon on the bottom right of the Main Screen.
3. Enter password (1,2,3,4) and press the key icon to access the Setup screen.
4. Press the ENABLE/DISABLE icon (first column, second icon). Consult page 5.14 for diagram of screen.
5. To disable an entire Module select the Module (1,2) with the Module Scroll up/down arrow and set the enable/disable switch to “0”.
6. To disable a Drawer first select the Module (1,2) that holds the drawer then select the Drawer (A,B,C,D) and set the drawer enable/disable switch to “0”. All the cells in that module will be disabled.
7. To disable a rack first select the Module (1,2) that holds the drawer, select the Drawer (A,B,C,D) that holds the rack then select the rack number and set the rack enable/disable switch to “0”. All the cells in that rack will be disabled.
8. To disable a cell first select the Module (1,2) that holds the drawer, select the Drawer (A,B,C,D) that holds the rack, select the rack number that holds the cell, select the cell number and set the cell enable/disable switch to “0”.
9. Press the CHECK icon to accept the changes.

10. To enable any disabled cells, follow the above procedures except change the enable/disable switch to "1".

QUALITY CONTROL

INDICATOR	FREQUENCY	ACCEPTABLE RESULTS	PROCEDURE	CORRECTIVE ACTIONS FOR UNACCEPTABLE QC RESULTS
Module Temperatures	Daily	Within acceptable range.	Read module temperatures on thermometers located in the module and record on Temperature QC sheet.	If internal thermometer reading differs +/- 0.5^{o} from the modules set point recalibrate the Module Temperature (Pg. 5.18 in Operator Manual). Complete QC Deviation Form.
Media Performance	Each shipment/lot of bottles	Acceptable appearance and sterility.	Retain the Certificate of Conformance for each lot of media (included cases of BacT/Alert media). Record results of appearance and sterility checks on Media Log.	Arrange replacement of any nonsterile bottles or those with unacceptable appearance.
Instrument Faults	Each occurrence.	NA	Record the fault code number, description of the fault and corrective actions taken on the BacT/ALERT Fault Log sheet.	Consult SYSTEM TROUBLE-SHOOTING section in the Operator Manual for resolution procedures and describe outcome on the BacT/ALERT Fault Log Sheet. If fault adversely affects a patient sample complete an INCIDENT REPORT.

QC Documentation:

1. Record temperature readings on BacT/ALERT Temperature QC sheets.
2. Document instrument faults on BacT/ALERT Fault Log.
3. Document corrective action on all unacceptable results on QC DEVIATION FORM.
4. Review QC results monthly.
5. Maintain a log of shipments of culture bottles and verify acceptable appearance, and sterility on MEDIA LOG.
6. Maintain copies of Certificates of Conformance for each culture medium documenting manufacturer's QC.

INSTRUMENT MAINTENANCE

1. Periodic preventive maintenance is conducted by bioMerieux according to schedule in service contract. Customer performed instrument maintenance is not required.
2. Document/describe in detail all instrument repairs on EQUIPMENT MAINTENANCE LOG.

DISINFECTION OF SPILLS WITHIN THE INSTRUMENT (5.2)

CAUTION: If spill is detected that might involve M. tuberculosis, only persons wearing protective equipment (gown and NIOSH N95 particulate respirator mask, or equivalent*) should remain in the room.

****Protective equipment is located in the laboratory.***

1. Visually inspect the extent of the leakage or spill to determine which cell, rack or drawer is contaminated.
2. Remove the leaking bottle if possible. If the bottled becomes lodged in a cell, call bioMerieux immediately. Do not pull on the rack to dislodge the bottle.
3. Unload/Reload bottles from affected rack(s). To relocate bottles into another available cell, rack or drawer go to the Main Screen and press CTRL+10 to enter a special Bottles Relocation mode. The indicator lights in loaded cells will be continuously lit, unloaded cells will be unlit. Pull bottles out <u>one at a time</u> and reload <u>one at a time</u> into any available cell. When all bottles are reloaded press the CHECK icon on the Main Screen.
4. Disable the affected cells, rack, and drawer.
5. If an affected cell contains a large amount of liquid, carefully remove with a pipette and dispose in biohazard waste container.
6. If spill is confined to one or a few cells of one drawer rack, the affected cells may be cleaned and disinfected with a 10% bleach solution as follows:
    a. Insert absorbent material (e.g. gauze) into the cell to remove any remaining fluid. Discard in biohazard waste container.
    b. Wipe out the interior of the cell with gauze saturated (but not dripping) with 10% bleach and leave for 15-20 minutes to decontaminate.
    c. Remove and discard gauze in biohazard waste container.
    d. Wipe the interior of the cell with gauze soaked in distilled water to rinse.
    e. Allow the cell to air dry.
7. Calibrate the cell (Consult Operator Manual 5.14)

8. If cell fails to calibrate, repeat cleaning procedure and recalibrate. If calibration fails again, disable the cell.
9. For more extensive spills it may be necessary to remove one or more racks or a drawer. (Consult Operator Manual 5.5 Removing a Rack or 5.8 Removing a Drawer.

CLINICAL APPLICATION

The BacT/ALERT 3D Microbial Detection System is an automated test system capable of incubating, agitating and continuously monitoring aerobic, anaerobic and mycobacteria media inoculated with patient specimens for the presence of bacteria, yeasts or mycobacteria. The instrument can detect positive cultures more rapidly than with manual methods and therefore provide critical information for patient management earlier.

TECHNICAL SERVICE

Hass Scientific & Medical Supplies, Nairobi, Kenya. Tel: 254-20-3864364, engineering@hasscientific.com.

REFERENCE

Operator Manual BacT/ALERT™ 3D, Version B.12, bioMerieux, Inc. Durham, NC.

APPENDIX A – BacT/ALERT Faults Log
APPENDIX B – Equipment Maintenance Log