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BLOOD CULTURE MANUAL

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INTRODUCTION

Although this section is mainly directed towards the processing of blood cultures, occasionally other specimen types (e.g. Sterile fluids, Bone marrow, abscess material) are received in blood culture bottles and thus their processing and work-up will be described in this section (Appendix V). The current blood culture system used in the Microbiology Laboratory is the non-radiometric BacT/Alert System manufactured by Organon Teknika.

Specimens collected using the BacT/Alert bottles must be accessioned and processed as quickly as possible upon arrival in the laboratory.

All reagents, kits and media **MUST** be quality controlled before use. All tests must include appropriate controls. (Refer to the Quality Control Manual).

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BLOOD

I. Introduction

Blood cultures are collected from patients with suspected sepsis or bacteremia. Virtually any organism may cause bacteremia. Thus, the isolation of all organisms from a blood culture must be considered significant and correlated with the clinical picture. At least 2 sets and no more than 3 sets of blood cultures should be collected from a patient with suspected bacteremia prior to the initiation of antimicrobial therapy. Collection of additional blood cultures may be indicated if the patient fails to respond to appropriate antimicrobial therapy or develops a new episode of fever or sepsis following an initial response to therapy. All sets of blood cultures received from a patient will be processed regardless of the number.

II. Specimen Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents/Materials/Media

See [Analytical Process - Bacteriology Reagents/Materials/Media List QPCMI10001](#)

IV. Procedure

See [Specimen Rejection Criteria QPCMI06001](#) to determine suitability of specimen.

A. Initial processing of BacT/Alert blood culture bottles

- i) Receive and/or order bottles in the LIS depending on location of origin; be sure to enter special instruction under "Order Comment" (e.g. SBE/IE, Fungus, FUO/PUO, etc).
- ii) Place specimen label(s) on bottle(s) leaving part of bottle bar code label (and the bottle bar code # if possible) uncovered. If the bottle bar code is not useable (due to damage or labels) replace it with the generic replacement label. **Note: Bottle type must be edited when loading to match the actual bottle type.**
- iii) Initial the bottle after attaching the specimen label to it.
- iv) Place one of the small LIS bar code labels on the original requisition, if one was received.
- v) Do not load bottles that are visibly positive (i.e. lemon yellow sensor disc, dark, hemolyzed or bulging septum) into the BacT/Alert System. Process these bottles as outlined in section B below. Change the bottle status to "Positive" under the DATA MANAGER option when the Gram stain and/or sub-culture becomes positive.

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vi) Special Requests:

1. Subacute Bacterial Endocarditis/Infective Endocarditis (SBE/IE) and Pyrexia of Unknown Origin/Fever of Unknown Origin (PUO/FUO)

Incubate bottles for 21 days in BacT/Alert.

Be sure to enter these requests under the “ORDER/ENTRY” comment field in LIS. Check the BC daily order comments printout form LIS to ensure all bottles needing 21 day cultures have been marked and “MAX TEST TIME” has been edited to 21 days under “Bottle Data” – DATA MANAGER.

2. Bone Bank Blood

Incubate bottles for 7 days in BacT/Alert.

Be sure to enter these requests under the ‘ORDER/ENTRY’ comment field in LIS. Check the Daily Bone Bank Printout to ensure that all requests for Bone Bank Blood cultures have been marked and “MAX TEST TIME” has been edited to 7 days under “Bottle Data” – DATA MANAGER.

3. Fungus and Yeast

Incubate bottles for 5 days in BacT/Alert.

4. Dimorphic Fungi (e.g. Histoplasma, Blastomyces and Cryptococcus)

If BacT/Alert bottles are received with a request for dimorphic fungi or cryptococcus, **notify the ward/physician** that they must use the Isolator 10 collection tubes. Process the BacT/Alert bottles as per routine blood cultures.

5. Brucella

Label bottle as “Brucella”. Incubate bottles for 21 days. Be sure to enter these requests under the “ORDER/ENTRY” comment field in LIS. Check the BC daily order comments printout form LIS to ensure all bottles needing 21 day cultures have been marked and “MAX TEST TIME” has been edited to 21 days under “Bottle Data” – DATA MANAGER.

6. Sterile Fluids

Incubate bottles for 5 days. See Appendix V for Handling of Sterile Body Fluids in Blood Culture bottles.

7. Bone Marrow (Sterility Testing)

Incubate bottles for 5 days. See Appendix V for Handling of Sterile Body Fluids in Blood Culture bottles.

vii) Incubate Blood culture bottles in the BacT/Alert System as follows:

Blood Culture	5 days	7 days	21 days
Routine (Blood, Sterile fluids, Bone Marrow)	X		
SBE/IE, PUO/FUO			X
Bone Bank Blood		X	
Fungus/yeast/Candida/Cryptococcus	X		
Brucella			X
Sterile Fluids	X		
Bone Marrow	X		

SBE/IE = Subacute bacterial endocarditis/Infective endocarditis

PUO/FUO = Pyrexia of unknown origin/Fever of unknown origin

B. Interpretation of BacT/Alert Blood Culture Bottles

The BacT/Alert System will continuously rock the blood culture bottles at 70 cycles per minute and scan all bottles (every 10 minutes) for evidence of growth. The machine will automatically flag any positive blood cultures. Process the blood culture bottles as follows:

a) Negative Cultures

- i) Routine, Bone marrow (for sterility testing), sterile fluids, and general fungus/yeast/candida/cryptococcus blood cultures:
Discard all negative bottles after 5 days incubation and issue a negative report.
- ii) Bone bank blood cultures:
Discard all negative bottles after 7 days incubation and issue a negative report.
- iii) SBE/IE and PUO/FUO, Brucella:
After 21 days incubation, discard all negative bottles and issue a negative report.

b) Positive Cultures

When the bottle has been flagged positive by the BacT/Alert or is macroscopically positive (i.e. bulging septum, obvious discoloration or lemon-yellow sensor disc) process **ONLY** the suspected positive bottle (do not sub-culture the matching bottle unless it is also macroscopically positive or flagged positive). If only one bottle has been flagged positive, leave the companion bottle in the BacT/Alert until completion of its incubation time, then discard if negative. Process all suspected positive bottles as follows:

- i) Gram stain
- ii) Sub-culture onto the following (whole) plates*:

Media	Incubation
Blood Agar (BA)	CO ₂ 35°C x 48 hours
Chocolate Agar (CHOC)	CO ₂ 35°C x 48 hours
MacConkey Agar (MAC)	CO ₂ 35°C x 48 hours
Fastidious Anaerobic Agar (BRUC)	AnO ₂ 35°C x 48 hours

* If a culture bottle marked as “Brucella” is flagged positive, remove a small amount of the culture for a Gram smear **ONLY**. If the Gram smear shows small gram negative bacilli, forward the positive culture bottle to the Public Health Laboratory (PHL) for identification, **DO NOT** subculture bottle. If the Gram smear shows organisms other than small gram negative bacilli, proceed to subculture the bottle with media as outlined above.

The Gram stain may indicate the need for additional media or a change in the incubation conditions. See the table below or the Charge technologist for appropriate additional media.

Direct tests and additional media for preliminary processing of positive BacT/Alert blood culture bottles:

Gram stain morphology	Direct test/Additional media
Gram positive cocci in clusters only	Thermonuclease
Gram positive cocci in pairs and chains only	Optochin disc, Bile esculin (BE)
Gram positive bacilli only	BE, motility if BE is positive (set up motility the next day if BE is read the next day)
Mixed Gram positive/Gram negative organisms	Add Colistin Nalidixic Agar (CNA)
Small Gram negative bacilli only	Add Campy Agar

Remove Positive blood culture bottles from the BacT/Alert and do not reload. Keep bottles in the Positive bottles tray until the isolate has been frozen and the final report has been issued.

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It is important to avoid inadvertently reloading true positive bottles without sub-culturing. If this was done by mistake, the bottle may not be flagged positive a second time. In BacT/Alert, this could result in a positive being reported as a negative.

Minimum work-up is performed for identification of isolates from **autopsy blood** specimens:

1. Single isolate culture:

Gram negative bacilli	Identify with Vitek (no sensitivities)
<i>Staphylococcus</i>	Pastorex Staph
<i>Streptococcus</i>	Bile esculin & PYR
	Streptococcus grouping

2. Mixed culture:

List organisms based on Gram stain morphology e.g.
“Mixed culture including gram positive cocci, gram positive bacilli and gram negative bacilli”.

c) False Positive Cultures

If the bottle has been flagged positive by the BacT/Alert and the Gram stain is negative, sub-culture as outlined for a positive culture above. Check the bottle graph in BacT/Alert. If the graph appears to be positive, recheck and/or repeat the gram stain and/or acridine orange stain. If the graph appears to be negative, enter the gram result "No bacteria seen" under media (GRAM). Do not assign an isolate #.

Reload the bottle under “LOAD BOTTLES” option. Scan the "bottle" barcode label only when reloading to BacT/Alert 3D. The bottle’s status will automatically be converted to “Unconfirmed positive – negative so far”.

Continue reading the plates. The culture will remain on the “BC Posted – No Iso” work list until the final BacT/Alert result is posted. (See section “Negative bottles”).

C. Processing of Sub-cultures

In the afternoon, examine the morning sub-cultured plates. Subsequently, examine plates daily for 2 days. Identification and susceptibility testing should be attempted as soon as there is adequate growth on a sub-cultured plate. Identify organisms as per routine and as outlined in the [Bacteria Work-up Manual](#) tables.

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If yeast is isolated, set up Germ tube. If germ tube is positive, report as "*Candida albicans*". If germ tube is negative, send a sub-cultured plate to Mycology bench for further work-up.

If both bottles of one set of blood cultures grow **morphologically identical organisms**, work-up only from one bottle of the set.

If multiple bottles from the same patient collected within 72 hours of each other are positive and growing **morphologically identical organism(s)**, perform complete identification and susceptibility testing from one set of the sub-cultured plates. For isolates from the other sets, perform minimal identification and oxacillin and/or vancomycin screens if the isolate is *Staphylococcus* or *Enterococcus*.

Examples of minimal identification:

- (1) Gram positive cocci in clusters: Perform Pastorex Staph only.
- (2) Gram negative bacilli: Perform oxidase test only and note if lactose fermenter (LF) or non-lactose fermenter (NLF).

Report the identification and refer the sensitivity results to the completely identified organism.

Note: If >1 morphologically distinct type of coagulase negative staphylococci (CNST) are identified, pool the isolates and perform susceptibility testing on the pooled sample. Report as a mixed culture with the sensitivity results of the pooled sample.

Blood Culture Isolates to be Frozen and Saved

Freeze **ALL** isolates from blood culture at -70°C **EXCEPT:**

- Enterococcus susceptible to Vancomycin
- Anaerobes
- *E. coli* susceptible to Amp and Septra
- Skin flora (CNST, Micrococcus, *Bacillus* sp., *Corynebacterium* sp. not JK, *Lactobacillus* sp., *Lactococcus* sp., *Propionibacterium* spp., *Peptostreptococcus* sp.
- Autopsy specimens
- Repeat isolates within 72 hours

Document freezing in Softstore.

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D. Susceptibility Testing

Refer to Susceptibility Testing Manual.

V. Reporting Results

Negative report:

Preliminary: The LIS will automatically report "Culture received in lab. Results will be reported as soon as they become available" and assign a preliminary status.

Final: i) Routine, yeasts, <i>Candida</i> , <i>Cryptococcus</i> Unspecified Fungus, sterile fluids, bone marrow (Sterility testing)	"No growth after 5 days incubation".
ii) SBE / IE, PUO / FUO	"No growth after 21 days incubation".
iii) Bone Bank bloods	"No growth after 7 days incubation".
iv) Dimorphic fungi	"No fungus isolated".

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Positive report:

For Gram stain results:

In LIS “Media” Window, under GRAMB media, pick from keypad:

1. The bottle type the organism was from e.g. from FO2.
2. Then pick the organism seen e.g. gram positive cocci in clusters
3. Then the isolate code to be transferred to the “ISOLATE” Window (if this is the first time this organism is seen in this order; omit this keypad pick if this is the second time this organism is seen in this order.)

Go to the “TEST” Window:

1. For Blood Culture test, **REMOVE** preliminary statement “Culture received in lab....” Add “Gram Stain: Gram positive cocci in clusters.....”etc.
2. Status the Test as preliminary (^P).
3. For fluids or aspirates in blood culture bottles report Gram results under the “ISOLATE” window of LIS as Isolates 1 "Gram positive cocci" etc. Status the test (C&S or FLDM) as preliminary (^P).

For all sites, telephone **the ward/ordering physician as soon as the Gram stain result is available**. If another bottle of the same set becomes positive with the same organism, no further report is required.

For Culture results:

Report organism with corresponding antibiotic susceptibility results (as appropriate).

If >1 coagulase negative staphylococcus (CNST) is identified and susceptibility testing is performed on the pooled sample, report as: "Coagulase negative staphylococcus, mixed growth" with the corresponding sensitivity results for the pooled sample.

For all sites, telephone the ward/ordering physician as soon as the Gram stain result is available.

If gram positive cocci identified and thermonuclease test is:

- i) Positive - LIS isolate field Report as “*Staphylococcus aureus*”. Isolate comment field "Presumptive identification confirmation to follow”.
NB: Remove isolate comment once it is confirmed.
- ii) Negative - No further telephone call is required.

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If a *S. aureus* is isolated (presumptive or confirmed) from a patient at the TTH, TWH or MSH (including the emergency department), notify the On-call Infectious Disease Physician immediately. Do not notify the On-call Infectious Disease Physician if the patient is deceased, was seen in an outpatient clinic other than the emergency department or is a neonate in the Neonatal ICU. Page the resident through TTH Locating (416-340-3155).

For identification and sensitivity results, call the results as soon as they become available as follows:

Hospital	Monday - Friday	Weekend / Holidays
TGH, TWH	No call*	No call*
PMH	No call*	Call
CHC, Ajax	No call*	No call*
MSH	Call	Call
Baycrest, TRI, CAMH, Grace	Call	Call

***Unless** a new organism is isolated that was not seen on the initial Gram stain, or the organism has been identified as *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Neisseria meningitides*, *Salmonella* species or *Cryptococcus neoformans*.

When both bottles in the set are completed, assign “Interim” status (^L). Senior staff will review and finalize the report.

VI. Reference

P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Tenover, R.M. Tenover. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

Guidelines for Routine Processing and Reporting of Blood Cultures for Bacteriology. 2003. QMP-LS Ontario, 1.2.1 p11-14.

S. Mirrett, M.P. Weinstein, L. Reimer, M.L. Wilson and B. Reller. 2001. Relevance of the number of positive bottles in determining clinical significance of Coagulase-Negative *Staphylococci* in blood cultures. J. Clin. Microbiol. 39:3279-3281.

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ISOLATER 10 BLOOD CULTURE SYSTEM FOR DIMORPHIC FUNGI

I. Introduction

The Isolator 10 blood culture system should be used for the isolation and detection of Cryptococcus and dimorphic fungi such as Histoplasma and Blastomyces.

If BacT/Alert bottles are received with a request for dimorphic fungi, notify the ward / ordering physician that they must use the Isolator 10 collection tubes. The BacT/Alert bottles should only be processed as per routine blood cultures.

II. Collection and Transport

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)

III. Reagents / Materials / Media

Isostat cap, Isostat concentration pipette, isostat supernatant pipette, 10% PVP iodine, Dupont Isostat, Vortex.

IV. Procedure

A. Processing of Isolator 10 Microbial Tubes:

1. Centrifuge specimen at 4700 rpm for 30 minutes.

NB: The use of a safety hood is mandatory for steps 2 to 9.

2. Disinfect the stopper using 10% PVP iodine or tincture of iodine. Leave for a few minutes. Remove excess iodine with alcohol gauze. Allow to dry completely.
3. Place cap over stopper. Grasp only the sides of the cap.

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4. Position cap under press and pull down handle and release.
5. Collapse bulb of supernatant pipette completely before inserting stem into the tube.
6. Insert stem into tube and release bulb to withdraw supernatant fluid. Discard the supernatant.
7. Vortex the tube for at least 10 seconds at the highest setting.
8. Collapse bulb of concentrate pipette completely and then insert stem into tube. Slowly withdraw all concentrate.
9. Dispense concentrate in a straight line along the surface of the agar. Keep inoculum away from the edge of the plate.

Media	Incubation	
Inhibitory Mold Agar (IMA)	O ₂ ,	28°C x 4 weeks
Esculin Base Medium (EBM)	O ₂ ,	28°C x 4 weeks
Blood Egg Albumin Agar (BEAA) x 2 plates	O ₂ ,	28°C x 4 weeks

10. Using the tip of the pipette, streak the plates. Use 15-20 passes perpendicular to the original inoculum line.
11. Dry plates, seal with parafilm and forward plates to Mycology for incubation and processing.

B. Interpretation of Fungal Culture Plates:

Refer to Mycology Manual.

V. Reporting Results

Refer to Mycology Manual.

VI. Reference

1. Isolator 10 Product Insert.

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APPENDIX I - Operation of the BacT/Alert Blood 3D Culture System

I. Overview

Bottles are tracked by the computer and must be loaded using the computer and barcode wand. Each bottle is read every 10 minutes by the machine. Positives are detected as growth-generated CO₂ which causes a colour change in the pH sensitive disc on the bottom of the bottle. The computer detects a positive by an increased rate of colour change, acceleration in the rate of colour change or a very high initial baseline reading. A minimum of 7 readings (70 minutes) is required before a positive is declared.

II. Logging-on / Password

A number of functions are available without logging-on. Buttons that are highlighted are available (e.g. Quick Data Entry).

- A. Touch “Log-on” button on the screen.
- B. Enter your password. This will allow access to the functions assigned to your password level. A few functions (e.g. System Configuration) will be available only to staff with higher level passwords.

III. Monitor Screen

This screen monitors a number of functions, including positive bottles, negative bottles and anonymous bottles which are ready to be unloaded. Warnings of problems also appear on this screen including instrument failures, interface failures, anonymous unloads, etc.

From the monitor screen a number of buttons take you to various functions:

A. Quick Data Entry

Used to edit source on bottles being loaded (e.g. Fluids, SBE blood, etc). Available without logging on.

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B. Data Manager

Used for editing, correcting and querying data in BacT/Alert data base. Divided into 3 data bases:

- i) Patient Data
- ii) Accession Data
- iii) Bottle Data.

C. Database

Used for data base maintenance (e.g. Back-ups).

D. System Log

Used to look-up problems in the system log book.

E. Configuration

Used to edit system configuration (e.g. Date/time, passwords, interface). Not available on "Normal Operation" passwords.

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APPENDIX II - Loading New Bottles

I. Venting

Venting is not necessary with the new generation of BacT/Alert bottles. Use 3% hydrogen peroxide to clean all blood from the exterior of the bottles before loading.

If an old generation aerobic adult or pediatric bottle is received (the old generation bottles are shorter than the new bottles), it must be aseptically vented before loading to enhance the growth of aerobic organisms. Vent the bottle in the laminar flow hood to minimize contamination. Thoroughly clean the rubber septum with 3% hydrogen peroxide followed by 70% alcohol gauze. Using a sterile venting unit or a sterile syringe with the plunger removed, pierce the septum and leave the needle in place for a few seconds until the vacuum in the bottle has dissipated.

II. Quick Data Entry

1. Touch the "Quick Data Entry" icon on the BacT/Alert terminal.
2. Wand in the accession # .
- 3.
4. Save

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III. Load Bottles

Note: when loading bottles, **always** handle only **one** bottle at a time to avoid loading errors.

1. Touch the blue “Load Bottles” icon at the BacT/Alert 3D controller screen.
2. Wand in the BacT/Alert bottle barcode. Check that the correct bottle type appears on the screen. If the wrong barcode was read (e.g. Accession #) or if the barcode scanner read the # incorrectly, the bottle type will display as “Unknown”. To correct this, touch the bottle bar code field to bring the cursor back and reread the bottle bar code correctly.
3. If a generic bottle bar code label was used to replace a missing or unreadable bottle barcode, the bottle type will display as “Unknown”. Touch the up/down arrows on the bottle type field to match the bottle being loaded.
4. After reading the bottle code, the cursor moves to the accession # field. Wand the accession #. Check to see that the accession # has been read correctly. The BacT/Alert will accept **any** barcode in these fields and will cause problems later if it has not been entered correctly.
5. If the bottle being loaded needs an incubation time other than the default 5 days, touch the “Maximum Test Time” icon (a bottle with a clock). This brings up the “Maximum Test Time” screen. Touch the arrow up/down to adjust the maximum test time (e.g. 21 days for SBE). Touch the “check” mark to save..
6. Drawers with available cells will have a green light on. Open the drawer and load the bottle in any cell with a cell light lit. When the bottle has been loaded, the bottle ID and accession # fields will clear and the BacT/Alert will be ready to scan and load the next bottle.
7. When all loading is complete, touch the “check” button to return to the main screen.

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APPENDIX III - Unloading Bottles

I. Positive Bottles

1. At the BacT/Alert terminal, touch the “Unload Positive Report” button. **You must generate this report before unloading the bottles or it will not be available.** When the report has printed, touch the “Unload Positive Bottles” icon on the 3D controller screen.
2. Drawers containing positive bottle(s) will now be indicated by a green light.
3. Open the drawer. Cells containing a positive bottle will have their cell light lit.
4. Continue unloading the positive bottles until the screen indicates that all positive bottles have been unloaded. (The # above the Unload Positive Bottles” icon will be zero.) It is not necessary to wand the bottles being unloaded because unloading the wrong bottle will trigger an immediate 911 “Invalid Cell Unload” warning.
5. If an incorrect bottle was unloaded, immediately reload it in the cell from which you removed it (the cell light will stay lit if you have not cancelled the warning message).
6. When all positive bottles have been unloaded, touch the “check” mark to return to the main screen.
7. Check unloaded bottles against "Unload Positive Report" to ensure they match. Initial and date the report. File the report within the Positive BC binder. If bottle does not match the list, investigate.
8. At the BacT/Alert terminal, touch the “Send Test Results” button. This will post the results to the LIS “BC Posted – No Iso” Worklist. If the other bottle in the set was previously positive, look on the “BC posted – with Iso” work list.
9. Find the positive accession on the appropriate work list, go to the media screen, cursor to the positive bottle, F8, and enter “BC +” (from the keypad) on the **line below** the “Positive” result from the BacT/Alert. This will generate the appropriate subculture media. F12 to the “Test screen” and generate subculture labels. Save the file. Do not give the order “Prelim” status at this time.

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10. Gram and subculture the positive bottle, remembering to write the bottle type and date sub cultured on the labels. Add one small barcode label to daily Positive Blood Culture list [BCdaylist.doc](#). When the gram stain is read and entered on the paper list, return to the appropriate LIS work list and enter the gram results under media (GRAMB). Enter the bottle type where the gram was performed on (from FO₂, ANAO₂, etc.). Add any needed tests or additional media at this time (e.g. Thermonuclease, BE, OPT, CNA, etc.). From the media screen, F7 to the isolate screen and enter the isolate number. Do not enter the “tested date” (tilde) at this time. F12 to the “Test” screen.
11. Under BC test line, enter the gram stain result from the keypad (>Pos BC). Assign the culture a “Preliminary” status. Before saving the file and exiting, be sure to view the report to check that it is correct. A hard copy may be printed at this time to facilitate phoning, faxing and entry into the freezer file and / or BacT/Alert System. Document calls and faxes under ^C.
12. Continue to work-up and incubate plates, reporting results as they become available. When a result becomes available (e.g. ID, sensitivity, be sure to enter the “Tested date” () and reassign the “Preliminary” status. When the positive bottle work-up is complete, it will remain on the “BC Posted – with Iso” work list until the matching bottle is finalized. At this time, enter the # of bottles positive (e.g. 1 bottle out of 2 as an “Isolate comment” (F7, F8, “6” >BLDC) and assign an “Interim” status to send the order to the “Review Work List” for review by senior staff.

False Positives

If the bottle has been flagged positive by the BacT/Alert and the Gram stain is negative, sub-culture as outlined for a positive culture above. Check the bottle graph in BacT/Alert. If the graph appears to be positive, recheck and/or repeat the gram stain and/or acridine orange stain. If the graph appears to be negative, enter the gram result "No bacteria seen" under media (GRAM). Do not assign an isolate #.

Reload the bottle under “LOAD BOTTLES” option. Scan the "bottle" barcode label only when reloading to BacT/Alert 3D. The bottle’s status will automatically be converted to “Unconfirmed positive – negative so far”.

Continue reading the plates. The culture will remain on the “BC Posted – No Iso” work list until the final BacT/Alert result is posted. (See section “Negative bottles”).

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II. Negative Bottles

1. All Blood cultures entered into the LIS that have not had a result posted from BacT/Alert will be automatically resulted by the LIS after receipt as “Cultures received in lab. Results will be reported as soon as they become available”.
2. Note that it is **not** necessary to actually load the bottles in the BacT/Alert to get this report! Be sure that any incorrectly entered blood cultures are cancelled and/or resulted (as appropriate) in the LIS and deleted from the BacT/Alert to avoid this. Consult with the Charge technologist if in doubt!
3. At the end of the incubation time (5, 7, or 21 days), the BacT/Alert will flag the finished bottles as “Negative”.
4. At the BacT/Alert terminal, touch the “Unload Negative Report” button to generate a list of negative bloods to be unloaded (if any).
5. At the BacT/Alert 3D controller, touch the “Unload Negatives” icon.
6. Drawers containing negative bottle(s) will now be indicated by a green light.
7. Open the drawer. Cells containing a negative bottle will have their cell light lit.
8. Pull out the bottles with a lit light beside it.
9. Continue unloading the negative bottles until the screen indicates that all negative bottles have been unloaded. (The # above the Unload Negative Bottles” icon will be zero.) It is not necessary to wand the bottles being unloaded because unloading the wrong bottle will trigger an immediate 911 “Invalid Cell Unload” warning.
10. If an incorrect bottle was unloaded, immediately reload it in the cell from which you removed it (the cell light will stay lit if you have not cancelled the warning message).
11. When all negative bottles have been unloaded, touch the “check” mark to return to the main screen.
12. At the BacT/Alert terminal, touch the “Send Test Results” button. This will post the results to the LIS “BC Posted – No growth” Worklist. If the other bottle in the set was positive previously, look on the “BC posted – with Iso” work list.

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13. For routine 5 day negatives, mark orders on the work list (F5) by matching with the BacT/Alert printout and batch result them using the “No growth – 5 days” MACRO. (This MACRO will include resulting all fluid C&S and FLDM tests as well).
14. For 7 day negative (the older cultures on the list-Bone Bank specimens). Mark them in the LIS worklist and press the "No growth - 7 days" MACRO.
15. Any bottles remaining on the BacT/Alert printout that were not found on the “BC posted - No growth” work list will be on the “BC posted – No Iso” worklist or the “BC posted – with Iso” worklist. These will be bottles which were in a set that contained one or more false or true positives.
16. Check any bottles remaining on the LIS “BC posted – No growth” worklist that were not found on the BacT/Alert printout. These will be bottles which were in a set that contained one or more false positives OR bottles that become negative after the printout was run. Result and send out as appropriate.
17. Sign and date the "Unload Negative Report". File this report in the Negative Blood folder.

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Approved by: Laboratory Director	Revision Date: December 17, 2004	

APPENDIX IV - Handling of Body Fluids in Blood Culture Bottles

Order/Entry:

1. PD effluent fluids that always arrive into microbiology in Blood Culture Bottles (majority are from UHN and CHC)

- At Order/Entry Screen, call up order by Auxiliary number.
- Add the test "Fluid in BC Bottle" (option I)
- Receive and plate as usual
- Adhere the specimen labels on the bottles (the labels with the lab number that contains the extension 93). **DO NOT** use the media labels or the specimen label for the C&S or FLDM test.
- Load the bottles to BacT/Alert 3D as usual by scanning the bottle barcode and then the specimen barcode.

2. Sterile Body fluids and Bone Marrow that are normally sent to the lab in a sterile container and do not require inoculating into blood culture bottles but arrive into the lab in a Blood Culture Bottle ONLY (order from UHN and CHC)

- At Order/Entry Screen, call up order by Auxiliary number.
- Add the test "Fluid in BC Bottle" (option I)
- Receive and plate as usual
- Before saving, press F7 to go to media screen. Cancel the media that are attached to the C&S or FLDM test and add the FO2 and ANAO2 media.
- Save the order and print the labels
- Adhere the specimen labels on the bottles (the labels with the lab number that contains the extension 93). **DO NOT** use the media labels or the specimen label for the C&S or FLDM test.
- Load the bottles to BacT/Alert 3D as usual by scanning the bottle barcode and then the specimen barcode.

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3. Sterile Body fluids and Bone Marrow that are normally sent to the lab in a sterile container and do not require inoculating into blood culture bottles but arrive into the lab in a Blood Culture Bottle ONLY (orders from MSH, TRI, Baycrest, Riverdale, Toronto Grace and CAMH).

- At Order/Entry Screen, enter demographics as usual
- At test order, select
 - N - "Fluids"
 - N - "Other Body Fluids"
 - O - "Fluid in BC Bottle"
- At Source Screen, select source
- Receive and plate as usual
- Before saving, press F7 to go to media screen. Cancel the media that are attached to the C&S or FLDM test and add the FO2 and ANAO2 media.
- Save the order and print the labels
- Adhere the specimen labels on the bottles (the labels with the lab number that contains the extension 93). **DO NOT** use the media labels or the specimen label for the FLDM test.
- Load the bottles to BacT/Alert 3D as usual by scanning the bottle barcode and then the specimen barcode.

4. Sterile Body fluids and Bone Marrow that are sent to the lab in a sterile container AND a set of blood culture bottles BUT only one order was placed in the HIS.

- Call the floor to place a second order.
- Process one order as usual without the blood culture bottle and it will be worked up on the Miscellaneous Bench.
- Process the second order as item 2 above on the Blood Culture Bench.

5. Sterile Body fluids and Bone Marrow that are sent to the lab in a sterile container AND a set of blood culture bottles AND two orders were placed in HIS.

- Process both orders separately, one as item 2. above and the other one as item 3. above.

6. Aspirates from non-sterile sites e.g. wound drainage, pus and abscess material that are normally sent to the lab in a sterile container but arrive into the lab in a Blood Culture Bottle ONLY.

- Treat the bottle as a specimen transport medium. Planting bench will inoculate the assigned media from aspiration of the bottle contents.

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7. Aspirates from non-sterile sites e.g. wound drainage, pus and abscess material that are sent to the lab in a sterile container AND a Blood Culture Bottle.

- Plant the specimen from the sterile container as usual and discard the blood culture bottle.

8. Ascitis Fluid in Blood Culture Bottles ordered in UHN HIS as “ASCBT” test.

- Receive the order in LIS as in routine Bacteriology orders.
- Save the order and print the labels
- Adhere the specimen labels on the bottles (the labels with the lab number that contains the extension 91).
- Load the bottles to BacT/Alert 3D as usual by scanning the bottle barcode and then the specimen barcode.

Resulting:

For Negatives

At 24 hours:

- From the "BCFLD" (New Fluid in Blood Culture Bottle) worklist, go to the specimens from the previous day and result both the ?BTLE and C&S or FLDM test as "~No growth to date, further report to follow" and status them as "Prelim" (a Macro is set up on each of the Blood Culture Benches to batch result these). At "GM" test result as "not applicable, specimen in blood culture bottle".

At 5 days:

- Generate printout of "Unload Negatives" before unloading negatives.
- Unload the bottles on that list.
- From the "BCNG5" (BC Posted - No growth) worklist, result the ?BTLE and C&S or FLDM test as "No growth after 5 days" and status them as "Final" (the macro BC Ng 5 days is set up to result both the Fluids and the Blood Cultures; there is no need to separately result the fluids).

For Positives

- Newly detected positives orders will appear on the "BCPSN" (BC Posted-No Iso.) worklist.
- After reading the Gram stain, from the test C&S or FLDM, go to the F7 Isolate screen and put in Isolate 1, and the Gram result under Org. ID field. Be sure that the TstID is

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C&S or FLDM. Press "" to enter the tested date. F12 to save and status the C&S or FLDM test as "Prelim".

- Go to the test line of ?BTLE, F7 to go to the Isolate screen and enter Isolate A. Be sure that the TstID is ?BTLE. F12 to save the test. Status "Prelim".
- Work up an isolate the next day from the "BCPOS" (BC posted with ISO.) worklist and the rest of the positive blood cultures.

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Approved by: Laboratory Director	Revision Date: December 17, 2004	

APPENDIX V - Handling of Bone Marrow in Blood Culture Bottles with Requisition from UHN

At UHN Clinical Desktop (Misys):

1. Log on to the UHN clinical desk top icon.
2. MISYS will come up. Log on.
3. Click All UHN.
4. Type in MRN e.g. 3154444 then press <Enter>.
5. Click on the appropriate visit.
6. Click on the selected visit.
7. Click Microbiology.
8. The “order generator” is the requesting physician. Choose the appropriate Dr.
9. Enter ‘n’ (now) regardless of when the specimen was actually taken.
10. Type is C&S. Enter. Select 1 from the list. <Enter>.
11. For Frequency, select 2 (regardless of when the specimen was actually taken).
12. For Specimen Definition: Scroll down the list until you find the appropriate number i.e. for Bone Marrow, choose number 21. <Enter>.
13. For Reason for Test, type in the site i.e. for Bone Marrow type in what is on the requisition. PB 1 Pre. Click OK.
14. OK (again)
15. For Date collected, type in date the specimen was actually taken. For today, type ‘T’. For yesterday, type T-1 etc. It is not necessary to put the time it was taken, but if you do it must be the correct format i.e. T-1 07:32.
16. For Collector, type in ‘U’ for unknown. <Enter> x 2.
17. Type in ‘A’ to accept order. <Enter>.

So you have just completed order entry, collecting and receiving all in one step. You should find the labels have printed in the planting reception area. Affix the HDS label to one bottle of each set.

Accessioning the Bone Marrows in LIS:

1. In the LIS go to Order Entry. Go to the end and enter the HDS number. <Enter>.

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2. Verify that this is the correct patient and specimen. Enter. Choose 'I' (fluid in Blood culture bottle). If there is **no** label on the requisition that says 'Vista', use the arrow up key to 'Report to' section and type in "SARAR KEAA1 CBSSC. If there is a 'Vista' label, skip the 'Report to' section.
3. Press '='. Check the source by pressing F7, to see that the correct bottles have been ordered. F1. <Enter>.
4. At 'Site', put the information from the requisition, i.e. PB 1 pre.
5. F6 x3. F12 x2. Y (yes you want labels.) Ensure the correct label printer is selected. <Enter> x 2.
6. Affix the soft label with the '93' number on them to each bottle.
7. Affix a small soft label to HDS requisition.

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APPENDIX VI - Blood Cultures From Ajax / Pickering Hospital

Only positive blood cultures are sent to this lab. Blood culture bottles have been incubated in their BacT/Alert incubator, have been flagged as positive gram stain has been performed and the ward has been notified. If the bottle was flagged positive after the normal carrier hours, a set of media plates would have to be sub cultured, incubated and sent along with the bottle.

The gram stain information will be written on a BacT/Alert printout containing the bottle identification. If no gram stain result was received call the lab at 905-683-2320 ext 1476 (days) x 1475 (evenings). File the Ajax printout into the dedicate binder.

PROCESSING PROCEDURE:

KEYSTROKES

INSTRUCTION / RESULT

At LIS

- | | |
|---|---|
| <ol style="list-style-type: none"> 1. "O " 2. End 3. 00BC0000####
BacT/Alert printout. | <p>For order entry, orders
To go to auxillary number field
Enter the Meditech order number from accompanying list or</p> <p>If "order not found", contact the LIS officer, DO NOT add an order in LIS. Order has to be ordered through Meditech for proper processing.</p> |
| <ol style="list-style-type: none"> 4. F12 ,
F6 x 3,
F12 x 2
Y | <p>To accept order and print labels</p> |

At BacT/Alert

5. Log on
6. "Quick data Entry"
7. Scan order number barcode
8. Type or scan bottle barcode number

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Issued by: LABORATORY MANAGER	Original Date: January 10, 2000	
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APPENDIX VII - Quality Control (QC) of the BacT/Alert System

Daily

When entering the “Blood culture Posted-No Iso” worklist, the LIS will prompt “QC pending: Would you like to bridge to QC?” Enter “Y”.

A. Incubator Temperature

- i) Enter each instrument temperature. Check the temperature first thing in the morning when it has stabilized for at least half an hour.
- ii) Swap zip diskette and enter “Done”.
- iii) Check the Bone Bank report to be sure all Bone Bank bloods have been edited to a maximum test time of 7 days.
- iv) Check the O/E Comment Report to look for orders from the previous day that may have comments that were missed when loaded. Look for requests for :
Brucella - bottle should be labeled “Brucella”.

Specific requests for Dimorphic fungi (e.g. Histoplasma, Blastomyces) – should be collected in an Isolator tube

SBE/IE, FUO/PUO – keep 21 days

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Issued by: LABORATORY MANAGER	Original Date: January 10, 2000	
Approved by: Laboratory Director	Revision Date: October 24, 2001	

APPENDIX VIII - Sterility Testing of Blood for Media

Initial testing (performed by blood culture bench)

On receipt in the laboratory, each bottle is assigned a letter (A, B, C, etc.). Aerobic BacT/Alert bottles are labelled with corresponding letters. The smaller portion of the barcode is attached to the original bottle of blood.

Enter the data in the LIS as follows:

MRN: 777777777

TESTS: BLOOD CULTURES

SOURCE: BFA

SITE: HORSE / SHEEP, LOT #, EXP DATE

With a needle and syringe, 2.5 mL of blood is aseptically transferred from each bottle of blood and inoculated into separate BacT/Alert bottles. The original bottles of blood are immediately refrigerated and the BacT/Alert bottles loaded and processed as routine specimens.

If any BacT/Alert bottle gives a positive reading, the QA technologist must be informed ASAP and the original bottle of blood is removed from use. The BacT/Alert bottle is Gram stained and subcultured to BRUC (AnO₂) and CHOC (CO₂). Identification to the species level (e.g. staphylococcus, diphtheroid, etc.) will be sufficient.

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Issued by: LABORATORY MANAGER	Original Date: January 10, 2000	
Approved by: Laboratory Director	Revision Date: November 16, 2001	

APPENDIX IX - Manual Back-Up Protocol

If the automated system should fail for >24 hours or if non-BacT/Alert bottles are received, the following procedures will be used.

1. Examine all bottles for macroscopic growth daily.
2. For macroscopically positive bottles, process as indicated under "Positive Cultures".
3. If macroscopically negative, sub-culture as follows:

Direct Examination: Not required.

Sub-culture:

Incubation Day	Bottle Type	Medium	Incubation
1	FO ₂ , O ₂ or PED	CHOC	CO ₂ , 35°C, x 48 hours
2 & 5	FO ₂ , O ₂ , PED or ANAO ₂	CHOC & BRUC	CO ₂ , 35°C, x 48 hours AnO ₂ , 35°C, x 48 hours

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Approved by: Laboratory Director	Revision Date: December 17, 2004	

APPENDIX X - Troubleshooting the BacT/Alert System

Troubleshooting

See BacT/Alert training manual and Troubleshooting Guide:

Service

For service call bioMerieux at 1-800-361-7321

Daily Problem Printouts from BacT/Alert

There are 7 reports generated from BacT/Alert to track problems that might have occurred during bottle feeding:

Report 1 - "Bottle with wrong accession number"

List of bottles that were loaded in the past five days that were linked to accession numbers that did not conform to the LIS accession number pattern.

Modification Procedure:

1. At BacT/Alert 3D press <CTRL> <F10>.
2. Pull problem bottle from its cell and note the LIS order number.
3. Go to 3D screen with user password.
4. Go to problem cell.
5. Move cursor to accession number area and type in the correct LIS number.
6. Exit 3D.

Report 2 - "Download problem with patient demographics"

Patient demographics were missing from an order in BacT/Alert. If the time BacT/Alert is performing certain functions, such as producing a report or daily back-up, coincides that of any LIS downloading an order, the order information will not be seen in BacT/Alert and will be tracked by this report.

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Modification Procedure

At LIS:

1. Go to 7, Interface
2. Go to "Download" ↵
3. F2
4. Select BacT/Alert
5. Use space bar to toggle to "All"
6. Change the date range if needed
7. F12 x 2
8. Move cursor down the list to the desired order number
9. <Enter>
10. Continue with other orders or F1 to exit
11. Check BacT/Alert by Accession No. to see if patient demographics has been transferred

Report 3 - "Accession number with no bottle"

This report will show a list of specimens that have not been received by BacT/Alert.

Modification Procedure:

1. Check the Report 1 and Report 4. The wrongly identified bottles may belong to the accession numbers on this report. If this is not the case, proceed to step 2.
2. Check LIS for "Received" and "Plated" date and time. If they were missing, this specimen has not been received in the microbiology lab. If the "collected" date and time is today, this is likely a specimen in transit and will arrive soon. If the "collected" date and time is older, inform a senior or charge technologist. It is possible that this specimen is lost in transit.

Report 4 - "Accession number with wrong bottle ID number"

This report list orders that were linked to bottle numbers that do not conform to the standard bottle codes. E.g. LIS order number or auxillary number were barcoded as bottle numbers.

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Modification Procedure:

1. At BacT/Alert 3D press <CTRL> <F10>.
2. Pull problem bottle from its cell and note the LIS order number and the bottle code.
3. Go to 3D screen with user password "1212".
4. Go to problem cell.
5. At the bottle code area, type in the correct bottle barcode. Also check the LIS number.
6. Press "√" mark.
7. This change will trigger an error code "932" in BacT/Alert 3D.
8. Press "X" to remove the warning screen.
9. Exit back to 3D main screen.
10. The bottle that was just modified will be noted as positive. Unload this bottle following the "unload positive" procedure. Subculture the bottle and reload the bottle following the "Load Bottle" procedure.

Report 5 - "Daily Status Report"

This is a list of all orders and bottles entered within the last 24 hours. Use this report as a guide for troubleshooting.

Report 6 - "Loading Problem Report"

This is a list of bottles that were loaded with proper bottle identification code but the Accession Number is missing, loaded within the last 24 hours.

Modification Procedure:

1. At BacT/Alert 3D press <CTRL> <F10>.
2. Pull problem bottle from its cell and note the LIS order number and the bottle code.
3. Go to 3D screen with user password "1212"
4. Touch the "Edit Data Relationships" button.
5. Enter the LIS number in the left "Accession Number" field that you wish to associate to the bottle.
6. Touch the bottle ID in the right "Bottle ID" scroll box to be associated with the accession number.
7. The selected bottle ID is highlighted.
8. Press the top "Data Transfer" button to move the Bottle ID to the left Bottle ID scroll box.
9. Press the "check" button to accept the changes.
10. Press the "Previous Screen" button to return to the 3D Main Screen.

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Report 7 - "Negative to Date Report"

This is a list of all bottles that were unloaded prematurely within the last 24 hours.

Modification Procedure:

1. Look for the "Negative to date" bottle from the hazard cardboard box for discard bottles.

If the bottle is found:

2. At BacT/Alert 3D controller Main Screen, touch the "Load" button.
3. Scan the bottle ID barcode only.
4. Reload the bottle into any unoccupied cell.
5. Write up an incident report.

If the bottle is not found:

2. Write up an incident report.
3. Refer the problem to the charge technologist or the designated responsible technologist for further investigation.

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Host Not Responding Message

When there is a problem with the interface between the LIS and BacT/Alert, a red box on BacT/Alert terminal will appear indicating "Host not responding".

Modification Procedure:

Restart Interface

(To re-establish LIS/BacT/Alert communication)

At LIS:

1. Go to **7. Interface** from main menu
2. **Setup**
3. Select **2 - Micro Instruments**
4. **Read**
5. **F12**
6. At "BactiAlert" **<Enter>**
7. **F7**
8. **F12 x 3**
9. **Quit**
10. Select **3 - AutoDownloading**
11. **Read**
12. **F12**
13. **F7**
14. **F12 x 3**
15. **Quit**
16. Select **3 - AutoPosting**

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17. **Read**
18. **F12**
19. **F7**
20. **F12 x 3**
21. **Quit**

Manual Downloading

Patient information not downloaded to instrument.

Modification Procedure:

1. Go to **7. Interface** from main menu
2. **Interface** menu
3. Select 1. BactiAlert
4. **Dloadlist**
5. Press space bar to **ALL**
6. Change Date Range to include problem order
7. **F12**
8. **F12**
9. Move the cursor to hi-light the order number
10. **<ENTER>**
11. Screen will display "downloading order #....."
12. Continue to hi-light another order number if required and repeat OR **F1**
13. **Quit**
14. **F1** to exit

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Manual Posting

Results from Instrument is not posted to LIS.
Modification Procedure:

At LIS:

1. Go to **7. Interface** from main menu
2. **Interface** menu
3. Select 1. BactiAlert OR 2. Vitek
4. **Open**
5. Select the date the testing was posted from the instrument
6. **Ledipost**
7. Move the cursor to hi-light the order number
8. **F8**
9. A red "P" will appear beside the order when completed
10. Continue to hi-light another order number if required and repeat OR **F1**
11. **Quit**
12. **F1** to exit

Restart Instrument

(If Downloading or Posting does not resolve problem):

1. Go to **7. Interface** from main menu
2. **Setup**
3. Select **2 - Micro Instruments**
4. **Read**
5. **F12**

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6. At "BactiAlert" <Enter>
7. **F7**
8. **F12 x 3**
9. **Read**
10. **F12**
11. At "Vitek" <Enter>
12. **F7**
13. **F12 x 3**
14. **Quit**
15. **Select 3 - AutoDownloading**
16. **Read**
17. **F12**
18. **F7**
19. **F12 x 3**
20. **Quit**
21. **Select 3 - AutoPosting**
22. **Read**
23. **F12**
24. **F7**
25. **F12 x 3**
26. **Quit**
27. Repeat Manual Dowloading or Manual Posting as needed.