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Section: Blood Culture Manual	Subject Title: Blood	
Issued by: LABORATORY MANAGER	Original Date: January 10, 2000	
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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.

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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

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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.

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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.