**UPMC Hanover Laboratory**

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| Subject: Positive Blood Culture Workup |  |

**PURPOSE:**

This document provides instructions for processing blood and body fluids flagged as positive by the BacTec FX blood culture system.

**SCOPE:**

This policy applies to UPMC Hanover Hospitallaboratory.

**PRINCIPLE:**

Blood culture vials loaded on the BacTec FX system are monitored for growth using photo detectors. When growth is detected, positive bottles are immediately flagged by an indicator on the front of the instrument and an alarm.

When a positive bottle is identified, it is removed from the instrument for identification, culture set up and susceptibility testing.

**SPECIMENS:**

This procedure applies to blood, pluoral fluid, peritoneal dialysate and ascites fluid.

**REAGENTS/SUPPLIES:**

Blood agar (BAP)

Chocolate agar (CHOC)

MacConkey agar (MAC)

BBE/LKV biplate

Brucella agar, pre-reduced (BRUC)

BioFire supplies

Sub-culturing/Venting unit

Frosted edge glass slides

Disposable Inoculating loops

**HAZARDS/PRECAUTIONS:**

* All positive blood cultures must be processed in the Biological Safety Cabinet (BSC).
* Do not remove slides for Gram stain from the BSC until they are completely dry.

**PROCEDURE:**

1. Print report of positive bottle, check Epicenter history of patient to determine number of bottles drawn and number positive. Record on printout.

2. Print specimen labels as needed. Place one on print out. Label plates (set up plates as listed below) and BioFire BCID (if indicated). Label 2 slides with 2 patient identifiers.

* Aerobic bottle BAP, CHOC, MAC
* Pediatric bottle BAP, CHOC, MAC
* Anaerobic BAP, CHOC, MAC, BRUC, BBE/LKV

3. In the BSC, wipe bottle top(s) with an alcohol prep, allow to air dry.

4. using the sub-culturing unit, prepare slides using one drop of blood per slide. Inoculate plates with one drop of blood per agar plate. Streak plates for isolation.

5. Incubate aerobic plates in the CO2 incubator. Place anaerobic plates in an anaerobic pack, seal and place in aerobic incubator #2.

6. Allow slides to dry in the BSC, then heat fix and Gram stain.

* Do not remove slides from BSC until dry.
* Do not dry slides on a heat block.

7. Review Gram stain

8. Set up BioFire BCID2 panel (if applicable) following the "Blood Culture Identification, BioFire BCID2 Panel" procedure found in MCN.

9. When set up is complete, place bottles in storage cabinet next to the BacTec.

Organisms seen on Gram stain

* Document Gram stain results and critical call documentation in EPIC.
* Order isolates and components in EPIC under culture work card.
* Second and third shift may omit ordering isolates and components in EPIC as long BacTec print out and Epicenter patient history report are held for day shift review.
* Call ED and Inpatient results within one hour to provider or nurse caring for patient.
* Outpatient results for Gram stain and BioFire must be called to primary care provider as soon as the BCID is complete.
* BioFire results for Inpatiens must be reported to "BioFire Team" in EPIC secure chat.
* Nursing home patient results for Gram stain and BioFire must be called to the nurse caring for the patient as soon as the BCID is complete.

No organisms seen on Gram stain

* Reload bottle in BacTec within 3 hours. Process inoculated plates following step #5 of procedure.
* If bottle flags positive a second time, Gram stain and review slide. If no organisms are seen, replace bottle in BacTec without subculture to plates. If Gram stain shows organisms, follow procedure above for positive bottle set up.

**INTERPRETATION OF RESULTS AND REPORTING: (Please review changes made JBM)**

Examination of plates:

1. If Gram stain, BCID result, and organism characteristics are consistent, set up identification/susceptibility testing.

2. All plates must be read at 24 and 48 hours.

3. If any discrepancies arise between plates, Gram stain, or BCID results, the discrepancy must be resolved.

a**. If organism does not match bottle Gram stain**, Gram stain colony from plate and review original Bottle Gram stain. If original Gram stain and plate Gram stain do not match, Re-stain and sub-culture from bottle.

b. **If organism does not match BCID results**, confirm ID through bench biochemical testing or Microscan combo panel. Culture bottle must be sub-cultured to compare with original plates to ensure that correct bottle was initially used to inoculate plates.

c. **If BCID result does not match Gram stain**, review bottle gram stain and/or re-stain second slide for review to confirm Gram stain results. If discrepancy still exists, make new slides from positively identified bottle and review Gram stain from new slides. If new Gram stain matches initial results, repeat BCID from positively identified bottle.

d. **See Microbiology supervisor for additional guidance if discrepancies cannot be cleared.**

4. Review results from Identity/susceptibility testing and correlate results with previous testing.

5. Preliminary verify if there are any outstanding negative bottles in set, or 48 hour plate reading has not been completed. Final verify if all bottles in set are positive, and 48 hour plate reading has been completed.

6. Possible contaminants must have the “Possible contaminant. For further work up please contact the Microbiology Department” comment entered under isolate, and possible contaminant flag placed under \*specimen update\*.

**Commonly Encountered Organisms:**

1. Staphylococcus aureus:

a. Identification is performed by BioFire BCID, latex agglutination, or Microscan Gram positive combo panel.

b. AST is performed on the Microscan Gram positive combo panel.

2. Coagulase-Negative Staphylococcus/Micrococcus sp.:

a. Reported as possible contaminant if recovered in only one set, unless only one set was collected.

b. Isolates of the same coagulase negative staphylococcus/Micrococcus found across 2 or more sets will receive definitive ID and AST by Microscan Gram positive combo panel or sent out for MALDI-TOF or reference lab if applicable.

3. Enterococcus sp.:

 a. Identification is performed by BCID, or Microscan Gram positive combo panel.

 b. AST is performed by Gram positive combo panel.

 c. Enterococcus sp. Will receive ID and AST if isolated from 1 or more sets.

 d. E. casseliflavus and E. gallinarum are intrinsically resistant to vancomycin.

4. Streptococcus sp.:

 a. S. pyogenes, S. agalactiae, and S. pneumoniae will all ID on the BioFire BCID panel. All

 other Streptococcus sp. Must be identified by Microscan Gram positive combo panel or

 sent out for MALDI-TOF.

 b. All beta-Streptococci and S. pneumonia will receive AST by Micro Strep 2 panel on

 the Microscan or by Kirby Bauer (See Kirby Bauer procedure for instructions).

 **Note:** S. pyogenes and S. pneumonia isolated from blood are auto-reported to the

 PA DOH through Epic.

 c. For only a single vial positive with Streptococcus species of the viridans group or alpha

 Streptococci not S. pneumonia, identify the isolate only. Do not perform AST. The viridans

group of Streptococci includes the following five groups, with several species within each

group: salivarius group, bovis group, anginosus group, (previously S. milleri group), and

mitis group. The anginosus group includes the small colony forming β hemolytic strains

with group A, C, F, and G antigens.

d. Non-S. pneumoniae alpha Streptococcus species isolated from only one bottle may be

 reported as viridans group if Gram stain is Gram positive cocci in pairs and chains,

catalase is negative, and PYR is negative. All alpha Streptococci that are PYR positive

must be sent for ID by Gram positive combo panel to rule out Enterococcus sp.

 e. Due to the possibility of Alpha Streptococcal endocarditis, All Non-S. pneumoniae alpha

 Streptococci isolated from 2 or more bottles must be identified and sent for AST.

5. Abiotrophia/Granulicatella species (Nutritionally variant Streptococci):

a. If the Gram stain from the vial shows pleomorphic Gram positive coccobacilli and only the

 chocolate subculture plate is growing, the isolate is suggestive of Abiotrophia and

Granulicatella. These organisms grow well on chocolate agar and are alpha hemolytic.

 b. These organisms are reported as Streptococcus, nutritionally deficient, with no AST

 performed.

6. Other Streptococcal-Like organisms: Aerococcus, Gemella and Pediococcus:

 a. ID is limited to bench biochemical and rapid testing panels. No definitive ID or

 AST to be performed unless seen in multiple bottles or by physician request.

 b. ID/AST is performed by Quest Diagnostics.

7. Coryneform Bacteria (Diphtheroids), including P. acnes:

 a. Coryneform bacteria are considered normal skin flora, and are reported as a possible

 contaminant, unless recovered from 2 or more bottles.

 b. ID/AST performed by request only, and is sent to Quest Diagnostics.

8. Listeria monocytogenes:

 a. L. monocytogenes is a small Gram positive bacillus/coccobacillus (sometimes mistaken

 as Gram positive cocci), and similar in colony morphology to group B Streptococcus.

 L. monocytogenes is a major concern in newborns, and should be identified as soon as

 Possible.

 b. All positive blood cultures that appear as small Gram positive bacilli/coccobacilli in blood

cultures obtained from newborns/infants should have a BCID panel set up for early detection of L. monocytogenes.

 c. L. monocytogenes can be presumptively identified by Grams stain (small Gram positive

 bacilli/coccobacilli), colony morphology (small smooth gray with narrow zone of hemolysis

 on BAP), positive catalase, motility at 25C, and positive rapid hippurate test. May also be

 identified by MALDI-TOF.

 d. Listeria monocytogenes isolated from 1 or more bottles must be sent to Quest diagnostics

 for susceptibility testing.

9. Bacillus species:

 a. Bacillus sp. Gram stain as large Gram positive rods, which may be chaining or contain

 endospores. Colonies are typically large flat and dry, and may be hemolytic or

 non-hemolytic on BAP.

 b. All non-hemolytic Bacillus isolates should be suspected for B. anthracis until further testing

 can be performed to rule out this agent of bioterrorism.

 c. All testing of isolates suspected of B. anthracis must be handled under a biosafety cabinet

 for plate reading and testing. Catalase testing should be avoided due to the chance of

 aerosolization of infectious material.

 Colonies of B. anthracis are non-hemolytic on BAP and may be mucoid or have a

 “Ground-glass” or “Medusa head” appearance. Isolates of B. anthracis are non-motile,

 and are sensitive to 10ug Penicillin disks. If B. anthracis cannot be ruled out, isolate

 should be sent to PA DOH for confirmation testing, following proper procedure for

 packing and shipping of category A substances.

 d. All non-anthracis Bacillus sp. can be reported as a possible contaminant, unless isolated

 from 2 or more bottles.

 e. Definitive ID/AST is not routinely performed unless found across culture sets, or by

 physician request. ID/AST is performed by Quest diagnostics.

**PROCEDURAL NOTES:**

Bacterial isolates are identified and susceptibilities performed as described in BioFire, MIC procedure and applicable identification procedures.

Fungus and yeast isolates are referred to Quest Laboratories for identification and susceptibilities as required.

Positive blood cultures for patients on comfort care and deceased patients is described in the document "Blood Culture Policy for Comfort Care and Deceased Patients".

**REFERENCES:**

Leber, Amy editor, Clinical Microbiology Procedures Handbook. 4th edition. American Society of Microbiology. Section 3.4.1.

UPMC Microbiology Procedure Manual. Workup of Positive Blood Culture Bottles. 9/20/2023.