Strong Memorial Hospital
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Document Title: Blood Culture Procedure Manual

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Positive Blood Culture station	1		

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CHANGE CONTROL FORM

Document Title:

1.Check one:

New procedure ☐	New process ☐	New form ☐	New flow chart ☐
Revised procedure x	Revised process ☐	Revised form ☐	Revised flow chart ☐
New job aid ☐	New labels ☐		
Revised job aid ☐	Revised label ☐		

2. Brief description of changes:

- Removed BacTAlert-specific information;
- Updated tests used to identify *Abiotrophia/Granulicatella*;
- Added all work-up of suspected *N. meningitidis* be performed in BSC;
- Changed method for identification of *Neisseria* spp.;
- Changed method for serogrouping *N. meningitidis*;
- Removed superoxol result for *M. catarrhalis*;
- Corrected glucose result for *S. paratyphi*;
- Clarified conditions for sending *Bacillus* spp. to NYSDOH to R/O *B. anthracis*;
- Yeast isolates frozen in Mycology;
- Test ciprofloxacin to predict fluoroquinolone resistance in *Salmonella* spp.;
- Clarified AST of yeast limited to *Candida* spp.;
- Specified that original GS result is not to be removed or over-written in LIS;
- Telephone results: updated latest definition of "ED", and who to contact for discharged patients.

3. S. O. P. validation needed? (Circle one) NO

4. Process validation needed? (Circle one) NO

5. Associated procedure and other documents (list those that need to be written or revised): None

6. Table of Content (TOC) update needed? (Circle one) NO

Author: Susan Raub Date: 6/6/2012
Reviewed by: Susan Raub Date: 6/6/2012
Approved by: Dwight J Hardy, PhD Date: 6/7/2012

BLOOD CULTURE PROCEDURE MANUAL

CONTENTS

BLOOD CULTURE PROCEDURE MANUAL

I. RECOMMENDATIONS FOR BLOOD CULTURES

A. Recommendations for Pediatric Blood Cultures

Pediatric blood culture practices often preclude multiple and large volume blood draws. Under these circumstances, the knowledge that pediatric patients with culture proven septicemia often have high numbers of organisms in the blood may obviate the need for large volumes. However, it should be noted that some pediatric patients with septicemia have low level bacteremia and there is no data to predict the value of a single negative blood culture. In addition, pediatric blood culture results from SMH and other medical centers indicate that obligate anaerobes are rarely isolated from pediatric patients and that some microbes which cause septicemia may not grow anaerobically. Based on this information, Clinical Microbiology and Pediatric Infectious Disease make the following recommendations:

1. At least 1 to 3 ml of blood should be obtained for culture whenever possible.

2. Since anaerobic bacteria are infrequent causes of pediatric septicemia and some organisms which cause septicemia may not grow anaerobically, pediatric blood should not be routinely inoculated into anaerobic culture bottles. Available blood should be inoculated into aerobic BacT/Alert bottles only.

3. Where clinical indications suggest infection with anaerobic bacteria, physicians will need to specifically request that blood be cultured anaerobically. In this case, available blood should be equally divided between anaerobic and aerobic bottles.

4. To aid in interpreting the significance of organisms such as coagulase negative staphylococci and viridans streptococci isolated from single bottle cultures, physicians are encouraged to consider ordering an additional blood culture(s). Each set of blood cultures should be obtained from a separate venipuncture to reduce the risk of contamination in multiple culture sets.

5. The ideal time interval between cultures has not been established but in general practice has been set at 15 to 60 minutes. In medically urgent situations, however, the time interval must be reduced to a few minutes so as to obtain specimens prior to initiation of antimicrobial therapy.

B. Recommendations for Adult Blood Cultures

Adult patients with culture proven septicemia often have few organisms, i.e., $\leq$1cfu per ml of blood. In addition, bacteremia in adult patients is frequently intermittent. Studies in adults have shown that predictive values of positive and negative blood cultures are increased as the number of cultures is increased from one to three. Based on this information Clinical Microbiology and Adult Infectious Diseases make the following recommendations:

1. At least 10 ml of blood should be routinely obtained from adult patients per blood draw when possible. Equal volumes of blood should be routinely inoculated into aerobic and anaerobic BacT/Alert culture bottles.

2. Two to three blood cultures should be routinely ordered for adult patients when possible. Each set of blood cultures be obtained from a separate venipuncture to reduce the risk of contamination in multiple sets.

3. The ideal time between blood cultures has not been established but in general practice has been set at 15 to 60 minutes. In medically urgent situations, however, the time interval between blood draws must be reduced to a few minutes so as to obtain specimens prior to initiation of antimicrobial therapy.

C. Guidelines for Isolator Blood Cultures

Culture of blood by a quantitative lysis-direct plating method using the Isolator System is significantly more labor intensive than the BacT/Alert system. In addition, the superiority of the Isolator system compared to other blood culture systems for the isolation of microbes has been documented only in selected cases. The Isolator system is recommended for patients where mycobacteria or *Histoplasma* are suspected agents of disease. Data from SMH indicate that two cultures using this method provide 100% sensitivity for the isolation of *M. avium* complex and *Histoplasma* during active untreated disease.

Use of the Isolator system for catheter-related sepsis is controversial. In patients with catheters, Isolators are not superior to routine culture methods for documenting sepsis from any source. When obtained in parallel from catheter ports and a peripheral venous site, Isolator blood cultures may be useful in discriminating between catheter-related and other sources of sepsis. The following guidelines for use of Isolator blood cultures are recommended:

1. Isolation of mycobacteria or *Histoplasma*. A maximum of two Isolator blood cultures per patient per week is recommended for isolation of mycobacteria or *Histoplasma*. Bacteremia or fungemia with these organisms is rarely seen except in AIDS. The Isolator system is not recommended for the isolation of *Candida albicans*, other *Candida* species, or *Cryptococcus neoformans*.

2. Documentation of catheter-related sepsis. Using the Isolator system with a single blood specimen from either a catheter or a peripheral vein is not useful since the interpretation of results is based on the comparison of the number of bacteria isolated from both specimens. Only one set of Isolator cultures is recommended for work-up of possible catheter-related sepsis. Isolator cultures for catheter-related sepsis should be drawn in parallel from a catheter port(s) and a peripheral venous site. Isolator tubes and accompanying requisitions must be labeled with the collection site, e.g., peripheral vein or type of line and color of port, to ensure that a comparison of quantitative bacterial counts can be made.

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From the Clinical Microbiology Newsletter, Univ. of Rochester Medical Center, Oct. 11, 2005.

II. Blood Cultures for Non-Fastidious Bacteria, *Candida species* and *Cryptococcus species*

A. Identification of Organisms on Positive Cultures

1. Staphylococci

a. General information about staphylococcal identification

1) Perform the latex agglutination and PYR test on all isolates of staphylococci. Make use of the Gram stain, catalase, and Microdase tests, as appropriate.

2) Invalid latex agglutination tests should be confirmed by a tube coagulase.

3) For abbreviated identification of isolates from second bottle of same set with same morphology, refer to chart on page 10.

b. Identification of *S. aureus*, *S. lugdunensis*

1) All staphylococci from blood cultures must be screened for *S. lugdunensis*.

Some strains may be latex agglutination positive (although they are tube coagulase negative) and can therefore be confused with *S. aureus*. Additionally, susceptibility testing is interpreted differently from other coagulase-negative staphylococci.

2) *S. lugdunensis* is PYR and Ornithine positive. *S. aureus* is PYR negative.

a) PYR negative: isolate is NOT *S. lugdunensis*. It may be another coagulase-negative staphylococci or it may be *S. aureus*. Report based on tube coagulase **and/or latex** testing. Any required susceptibility testing may be performed by the Vitek.

b) PYR positive: isolate is NOT *S. aureus*; it MAY be a *S. lugdunensis*.

i. Inoculate a Rapid Ornithine test, cover with oil. Incubate in non-CO_2. Check at approximately 2 hours to see if broth has turned yellow. Read at 4 hours. If negative at 4 hours, reincubate overnight.

-- Purple Color = positive.

S. lugdunensis is often positive within the 4 hour time frame, but may take longer.

-- Yellow, Brown-yellow, or straw = negative.

Isolate is most likely another species of coagulase-negative staphylococci.

ii. Send an isolate of *S. lugdunensis* to Vitek for susceptibility testing.

2. Alpha hemolytic Streptococci (AHS)

Perform DOC (or optochin (P) disc) on all alpha hemolytic streptococci.

a. *S. pneumoniae* -

Alpha hemolytic, Gram-positive cocci, which are catalase negative, DOC positive, and optochin (P) disc sensitive.

b. *Abiotrophia/Granulicatella* species (Nutritionally-variant strep)

"Alpha" hemolytic streptococci on Chocolate agar (No growth on blood agar), **LAP weak positive (pink) or strong positive (red), PYR wk + or (-), esculin (-)**

In addition, swab BAP agar with isolate to achieve confluent growth. Place a 2" streak of *S. aureus* across inoculum.

Abiotrophia sp/Granulicatella sp. (Nutritionally-variant streptococci) will only grow around the Staph streak.

c. Other Alpha hemolytic streptococci

Test alpha hemolytic colonies using the presumptive identification scheme (BANC). If the original Gram stain from the bottle does not show characteristic *Streptococcus* morphology, repeat the Gram stain from the growth on solid media.

Screen for *Enterococcus* sp. (see 3. below)

Speciation of streptococci is performed on special request only. If requested, record name of requesting physician. Also attempt to determine the question being asked; e.g. Are all isolates on a given patient the same species or is the concern that the isolate is a species considered more resistant than most?

Discuss with supervisor and prepare isolate for sending to New York State Dept. of Health Laboratory.

For identification of isolates from second bottle of same set with same morphology, refer to chart on page 11.

3. Gamma hemolytic streptococci

   a. *Enterococcus species*

      Perform PYR/ESC or BANC on gamma or alpha/gamma colonies.

      PYR/ESC +/+ or BANC +-+-: report as *Enterococcus species*

   b. Nonenterococcal gamma hemolytic streptococci

      PYR/ESC +/- , -/-, or -/+ - perform BANC

      BANC + - - - send isolate to Vitek for identification (rule out *S. bovis*)
      Vitek percentage ≥ 85% for *S. gallolyticus/pasturans* – report as *S. bovis*
      Vitek percentage <85% consult supervisor for reporting

      NOTE: screening for *S. bovis* is done whether pure or mixed culture.

      BANC - - - -: report as "Gamma strep not Group D"
      BANC - - - +: probable non-hemolytic Group B - do latex to confirm.

      Speciation of streptococci is performed on special request only. If requested, record name of requesting physician. Also attempt to determine the question being asked; e.g. Are all isolates on a given patient the same species or is the concern that the isolate is a species considered more resistant than most?

      Discuss with supervisor and prepare isolate for sending to New York State Dept. of Health Laboratory.

4. Beta hemolytic streptococci

   Perform serological grouping as soon as possible. **Consult Master Reporting Chart.**

5. Gram-negative diplococci

   Whenever *Neisseria meningitidis* is suspected to be growing, all work should be performed in a biological safety cabinet.

   Identify by Gram stain, oxidase, Superoxol. Additional testing may include **Carboferm Neisseris kit and Rapid NH**. For abbreviated identification of isolates with same morphology from second bottles of same set, refer to page 10.

   a. *N. meningitidis*

1) Should be Gram-negative diplococci, oxidase positive and Superoxol negative (or delayed positive).

2) **Perform CarboFerm Neisseria Kit.** Consult a supervisor. **Consult Master Reporting Chart.**

3) Serotype by slide agglutination in groups A, B, C and Y antisera **using direct inoculation of the organism into each antisera group**. Isolates that fail to type are sent to the NYSDOH laboratory for serogrouping.

b. *Neisseria gonorrhoeae*

1) Should be Gram-negative diplococci, oxidase positive and Superoxol strongly positive.

2) **Perform Carboferm Neisseria Kit.**

3) **Rapid NH can be performed if biochemical testing is inconclusive.** Consult a supervisor.

c. *Moraxella catarrhalis*

1) Should be Gram-negative diplococci, oxidase positive, **opaque, waxy. Perform CarboFerm Neisseria kit.** Consult a supervisor.

d. Other Gram-negative diplococci

1) Other *Neisseria* should be Gram-negative diplococci and oxidase positive. They may be pigmented.

2) Subculture to Martin-Lewis selective media. Non-pathogenic *Neisseria* will not grow.

3) **Perform CarboFerm Neisseria Kit or Rapid NH.** Results may not be conclusive. Consult a supervisor.

4) Other organisms with similar morphology include *Kingella* spp., other *Moraxella* spp., or *Acinetobacter* spp. Discuss with a supervisor.

Report as *Neisseria* species, not *N. meningitidis* or *N. gonorrhoeae*

6. Gram-negative bacilli

NOTE: For abbreviated identification of isolates with same morphology from second bottles of same set, refer to chart on page 11.

a. Subculture of anaerobic bottle fails to grow aerobically after 18-24 hr of incubation

1) Subculture bottle anaerobically to COL and KV media. If suspicious of *Fusobacterium*, also subculture to LLA.

2) Attach the following isolate comment to the 'Gram-negative bacilli" report:

"No growth on aerobic subculture. Organism presumed to be anaerobic. Identification to follow"

3) Call the patient location to notify them of the updated report. This will facilitate changes in therapy that may be necessary.

b. Enterobacteriaceae

1) Full identification is done on each colony type by Vitek or API. Complete speciation of a Gram-negative bacillus is done unless physician or supervisor discontinues workup. If more than one set has the same organism according to preliminary tests, complete workup (susceptibilities, freezing) can usually be done on one isolate per collection date only.

2) *Salmonella species*

Salmonella species are identified biochemically and serologically. Biochemical identification must be ≥85% from Vitek or at least very good ID from API. All negative lysine results from Vitek or API must be confirmed with a conventional lysine. Report as "*Salmonella* serogroup ___" with these exceptions: *S. typhi, S. paratyphi A, S. cholerae-suis.* Criterion for identifying each of these organisms follows.

S. cholerae-suis	*Salmonella* by API or VITEK. Types in group C. (Note: All group C are not *S. cholerae-suis*). Set up additional biochemicals listed below.
S. paratyphi A	*Salmonella* by API or VITEK. Types in group A antisera. Set up additional biochemicals listed below.
S. typhi	*Salmonella* (usually *S. typhi*) identified by API. Types in group D and Vi antisera. It may be necessary to boil the organism in order to obtain agglutination in D.

Salmonella D Types in Poly and D. Vi is negative
Report as *Salmonella* Group D, not *Salmonella typhi*

TEST	*S. cholerae-suis*	*S. paratyphi A*	*S. typhi*	Other
Arabinose	- (0)	+ (100)	- (2)	+*
Citrate	V(25)	- (0)	- (0)	+*
Glucose (gas)	+ (95)	**+ (99)**	- (0)	+
Lysine decarb.	+ (95)	- (0)	+ (98)	+
Ornithine decarb.	+ (100)	+ (95)	- (0)	+*
Rhamnose	+ (100)	+ (100)	- (0)	+*
Trehalose	- (0)	+ (100)	+ (100)	+*

( ) indicates % positive
* *Salmonella pullorum* and *Salmonella gallinarum* have variable results. Refer to ASM charts.
Adapted from TABLE 1 (Biochemical reaction of the named species, biogroups, and Enteric Groups of the family Enterobacteriacae) Clinical Manual of Microbiology, 9th edition.

c. Non-fermenting Gram-negative bacilli

1) *Pseudomonas aeruginosa*: may use abbreviated identification scheme if organism is:

a) Oxidase positive, Gram-negative bacilli exhibiting colony morphology which is flat and spreading with a metallic sheen, have grape-like or corn taco odor, AND

b) Produces a diffusable green or blue-green pigment on Mueller-Hinton agar.

The above reactions MUST be OBSERVED and CONFIRMED by a checker or supervisor.

c) If the results do not meet the criteria described above, a complete identification using the Pseudomonas screen (oxidase, 42°C, Pseudocel and OFglucose, OF maltose) or Vitek must be performed. *P. aeruginosa* identified by Vitek is acceptable to report.

2) *Stenotrophomonas maltophilia, Acinetobacter* ***baumannii,*** *Burkholderia cepacia and Achromobacter xylosoxidans*

Identification can be accepted from Vitek if the percentage is ≥85%.
<85% identifications require further conventional biochemicals.

3) *Haemophilus species*

a) Test for X and V requirements using the Haemophilus Quad plate. Rapid NH biochemicals may be needed to aid in speciation of *Haemophilus* other than *H. influenzae* and *H. parahemolyticus*.

b) Type in *H. influenzae* type b antisera
(Use *H. influenzae* from Kirby-Bauer station for positive control)

c) Report results of typing as soon as possible.

d. Gram-negative bacilli other than Enterobacteriaceae, *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter* and *Haemophilus influenzae* should be discussed with a supervisor.

7. Gram-positive bacilli – refer to the Identification of Gram-positive Bacilli protocol for additional details.

a. *Listeria monocytogenes* – Perform the Gram-positive rod screen which should have the following test results:

Test	Results
Gram stain	Gram-positive bacilli
Catalase	positive
Oxidase	negative
Esculin	positive
Motility	positive (umbrella pattern in semi-solid motility medium)
CAMP	enhanced hemolysis

If the isolate is consistent with *Listeria* species perform Coryne API. Refer to the Identification of Gram-positive Bacilli protocol for information on the API procedure, interpretation of results, and additional testing which may be needed.

b. *Corynebacterium jeikeium* – perform the Gram-positive rod screen which should have the following test results:

Test	Results
Gram stain	small, Gram-positive coccobacilli
Catalase	positive
Oxidase	negative
Esculin	negative
Motility	negative
Growth rate	better on BAP than CAP

If the isolate is consistent with *C. jeikeium* perform Coryne API. Refer to the Identification of Gram-positive Bacilli protocol for information on the API procedure, interpretation of results, and additional testing which may be needed.

c. *Bacillus species* – refer to the protocols: Culture and Identification of *Bacillus anthracis* From Clinical Specimens and Identification of Gram-positive Bacilli for details of identification.

1) Identification is based on Gram stain, spore stain, ß-hemolysis and motility. If the organism is ß-hemolytic, it is NOT *B. anthracis* and may be reported as *Bacillus* species, not *B. anthracis* when spore formation has been demonstrated.

2) If the isolate is large, white, irregular and non hemolytic, bring to the attention of a supervisor before proceeding. Motility (S) is set up. If the organism is a motile spore-former, report the organism as "*Bacillus* species, not *B. anthracis*. If the motility is negative **and the isolate is non-hemolytic**, send isolate to the state for further identification.

d. *Propionibacterium* species

Identification is performed by the Anaerobe laboratory

e. Branching Gram positive bacilli

All branching Gram-positive bacilli should be discussed with supervisor. They should be sent to the Anaerobe Lab and /or the Mycology Lab (depending on the bottle involved and growth characteristics) for identification.

f. Other Gram-positive bacilli

For detailed information refer to Identification of Gram-positive Bacilli Protocol.

1) If an isolate on Gram stain is a **pleomorphic** Gram-positive bacilli that resembles a *Corynebacterium* sp. (diphtheroid), is catalase +, oxidase -, esculin -, and motility -, report the organism as "*Corynebacterium* species (Presumptive identification)"

2) If an isolate on Gram stain is a pleomorphic, Gram-positive bacilli (**not typical of *C. jeikeium***), or a regular Gram-positive bacilli and is catalase – **and** esculin + , report as "Gram-positive bacilli, not *Listeria* or *Corynebacterium* sp., probable skin contaminant."

8. Rapid-growing Acid-fast bacilli – refer to Mycobacteriology Laboratory.

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9. Yeast - Identification is performed by the Mycology Laboratory.

10. Identification for additional positive bottles - same patient, same collection date.

    a. Identify one isolate of each morphological type per set according to routine procedures. Refer to chart below for abbreviated identification of isolates from other bottles of the same set with the same morphology.

    b. If there is any doubt in calling isolates the same on basis of tests indicated on the chart and colony morphology, perform complete identification.

    c. Cultures with multiple isolates in multiple sets or unusual isolates should be discussed with a supervisor.

Organism to be identified	Isolation of other bottles of same set with same morphology
Staphylococci	Latex agglutination
Streptococci	Same hemolysis; DOC on alpha
Gram-negative diplococci	Oxidase
? *Haemophilus*	Growth on CAP; no growth on MAC and BAP
LF	LF on MAC; spot indole
NLF	NLF on MAC; spot indole; oxidase
Gram-positive bacilli (small, aerobic)	Catalase, Gram stain
Yeast	Subculture of each bottle to Mycology

11. Positive blood cultures with ≥ 4 organism types may be reported descriptively with no further work-up. All isolates must be frozen. Add a comment to contact laboratory if further work-up is warranted.

12. Save Isolates

    a. All isolates are frozen at –70°C in TSB with 15% glycerol as soon as possible. They are stored for one year. **Yeast isolates are frozen in Mycology.**

b. Save one of each colony type of isolate from each day the patient's blood was drawn. N.B. Different antimicrobial susceptibility patterns are indicative of different strains.

13. Save positive bottles

Bottles positive by Gram stain and/or culture are held for 1 week.

B. Antimicrobial Susceptibility Testing

1. General Information

a. If organism does not grow adequately for Vitek and/or Kirby-Bauer add the following comment in the isolate comment field:

"Failed to grow adequately for routine susceptibility testing"

b. MIC's of most organisms are done on request only with approval of Infectious Disease Service. For some organisms MIC susceptibility is the routine method (ex. *S. pneumoniae*, alpha hemolytic strep) and does not require a request.

2. Staphylococcus species

a. *S. aureus*

One isolate, per collection date is tested.

Penicillin susceptible *Staph aureus* needs further testing. Send to MIC's. Refer to Kirby-Bauer protocol for further information.

b. *S. lugdunensis*

One isolate per collection date is tested.

c. Staph species coagulase negative other than *S. lugdunensis*

ONLY when there has been >1 positive culture (including Isolators) within a 3 day period AND it is a pure culture (2 colony types of STAN is considered a pure culture until proven otherwise). Separate collection sites (Broviac and peripheral) require separate susceptibilities.

3. *Strep pneumoniae*

Send isolate to MIC's for penicillin and ceftriaxone. One isolate, per collection date is tested. Repeat testing is not performed within 3 days.

4. Alpha hemolytic Strep

   Isolates in pure culture are sent to the MIC lab for susceptibility testing only if there is >1 positive culture (including isolators) within 3 days. Repeat testing is not performed within 3 days.

   N.B. Consider multiple colony types of alpha (or gamma) strep a pure culture. No comment is included in a mixed culture report.

5. *Enterococcus species*

   One isolate, per collection date is tested.

   Isolates are sent to MIC's for testing against daptomycin only on request. Repeat testing is not performed within 3 days.

   If the isolate is penicillin-susceptible, vancomycin-resistant, perform a Vitek identification, and if the isolate proves to be *E. faecalis* or *E. casseliflavus/gallinarum*, sign the isolate out as *Enterococcus* sp., not *E. faecium* (entnef).

6. Non – Enterococcal gamma strep and Gamma Strep not Group D

   Add the following isolate comment (&BSID):

   "If susceptibility tests are needed, please contact the on-call Infectious Disease physician".

   N.B. Consider multiple colony types of alpha (or gamma) strep a pure culture. No comment is included in a mixed culture report.

7. Beta hemolytic streptococci

   Routinely send to Kirby Bauer station for susceptibility testing and D-test to erythromycin and clindamycin. One isolate per collection date.

8. *Haemophilus influenzae*

   Perform ß-lactamase testing

9. *Moraxella catarrhalis*

Perform ß-lactamase testing.

10. *Neisseria species*

No routine susceptibility testing is performed

11. Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter species*

Perform Vitek susceptibility testing. One isolate, per collection date is tested.

Note that any isolate of *P. mirabilis* not susceptible to **aztreonam and/or** ceftriaxone must be sent to MIC's for confirmation of ESBL production.

12. *Salmonella species*

Perform Vitek and Kirby-Bauer susceptibility testing. One isolate, per collection date is tested.

Ciprofloxacin results must be obtained by disk diffusion to test for reduced susceptibility to fluoroquinolones (Refer to Kirby-Bauer protocol)

The aminoglycosides and the 1st and 2nd generation cephalosporins will be suppressed from reporting.

13. *Stenotrophomonas maltophilia, Burkholderia cepacia,* and *Aeromonas hydrophila*

Perform Kirby-Bauer susceptibility testing. One isolate, per collection date is tested.

14. Other Gram-negative bacilli

No routine susceptibility testing is performed.

15. Gram-positive bacilli

No routine susceptibility testing is performed

16. Yeast

Susceptibility testing will be performed by E-test in the MIC laboratory on **species of *Candida*.** The antifungal agents will include one or more of the following: fluconazole, voriconazole, caspofungin.

III. Blood Culture for Other Bacteria, Dimorphic Fungi, *M. furfur*, Mycobacteria

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A. Bacteria

1. Culture-negative endocarditis (including HACEK organisms)

a. Request

BacT/Alert bottles may be held on the instrument for 14 days and subcultured on Day 14.

Ask and note in the LIS and BacT/Alert the following information:
- What organisms are of concern?
- Name of physician making the request AND his/her pager number.
- Which culture sets should be held (maximum of 3 aerobic bottles per patient)
- Change the status to Interim
- Mark each bottle to be held with a dot on the top of the bottle indicating "HACEK"
- Change test time from 5.0 days to 14.0 days in Observa

b. Special Hold Subculture List

Place patient accession label on the form, select the reason, and indicate the date each bottle is to be subcultured.

c. Subculture of BacT/Alert bottles

On Day 14, Gram stain and subculture approximately 0.1 ml from each aerobic bottle to chocolate agar and Brucella blood agar plates. No anaerobic subcultures are performed. Incubate all plates 3 days at 36°C in CO_2.

d. Isolators - refer to Isolator protocol.

2. Isolation of *Brucella*

a. Recommend Serology.

b. Suggest use of Isolator in addition to BacT/Alert bottles.

NOTE: All work with specimens or cultures suspected of harboring *Brucella* should be performed in a biosafety cabinet.

c. Processing

1) Isolators: Four (4) Brucella blood agar plates (BBA) and one CAP are inoculated and taped with gas permeable tape and incubated at 36°C in CO_2 for up to 7 days.

2) Bottles: Read on the machine for 14 days. Subculture on Day 14 to BBA and CAP. Tape with gas permeable tape and incubate at 36°C in CO_2 for up to 7 days.

d. Culture Work-up

1) *Brucella sp.* are small (minute), slow-growing, faintly staining, Gram-negative coccobacilli.

2) Biochemical characteristics:

oxidase positive
catalase positive
nitrate positive
indole negative
urease positive
motility negative

3) Consult a supervisor is *Brucella* is suspected.

3. Isolation of *Bartonella* (cat scratch disease)

a. Recommend Serology.

b. Recommend Isolator.

1) Four (4) Brucella blood agar plates (BBA) and one CAP are inoculated, taped with gas permeable tape and placed in a candle jar (add a moist paper towel).

2) Read for growth at 1 week, 2 weeks, 4 weeks and a final read at 6 weeks.

3) *Bartonella sp.* grows as smooth small gray-white colonies on CAP. Colonies may be adherent. Gram stain may show curved weakly staining Gram-negative bacilli. The organism possesses twitching motility, and is catalase and oxidase negative. Any suspicious growth should be brought to the attention of a supervisor.

4) Negative cultures are reported as: "No *Bartonella* isolated"

4. Isolation of *Streptobacillus moniliformans*

a. Processing

1) Blood must be sent in a blue-top tube with sodium citrate.

2) Using the Isolator centrifuge, spin the tube at 4700 rpm 30 minutes.

3) Plate the sediment onto 2 sets of BAP and BBA.

4) Incubate one set aerobically at 36°C, CO_2, and one set in anaerobic chamber.

b. Culture Work-up

1) Hold plates for 1 week

2) Colony morphology and Gram stain – grey butyrous colonies that stain as filamentous, bulbous (swollen sections) Gram-negative bacilli.

3) Biochemical Reactions

catalase - negative
oxidase - negative
indole - negative
urea - negative
arginine with serum supplement - positive
nitrate - negative
OF glucose and OF maltose - weak positive

4) Consult supervisor before reporting.

B. Isolation of Yeast /Fungi

Person receiving an unclear request, either verbal or written, should determine, through conversation with the physician, what will be done. If the request is for fungus, determine whether for yeast or for dimorphic fungus, (i.e., *Histoplasma*) or for *Cryptococcus*. Refer to following page for specific information.

Note in the LIS the outcome of the conversation and the name of the physician contacted.

1. Culture for *Malessezia furfur*

a. Request

1) Prospective request ... recommend Isolators.

- 1 pediatric or adult tube drawn through the line
- 1 from a peripheral vein

2) Retrospective request ... subculture BacT/Alert bottles as soon as possible after request is made. Add appropriate media and the request for subculture in the media field.

b. Subculture of BacT/Alert bottles

1) Subculture all sets, especially if drawn through a line. If there are many sets, discuss with supervisor.

2) Preparation of Plates

- Place 2 drops of sterile olive oil onto SAB with pipette (a tube of sterile olive oil is found at the Isolator Station.)
- Spread over surface of plate using swab (as for Kirby-Bauer)

3) Remove 0.1 - 0.2 ml from aerobic bottle(s) and streak out (as for urine) on SAB with olive oil.

4) Incubate plates at 36°C (Blood station CO_2 incubator) for 5 days, reading daily. Send to Mycology if there is any growth.

5) Continue to read bottles for total of 5 days on BacT/Alert.

c. Isolators - refer to Isolator protocol.

2. Cultures for Dimorphic Fungi or Mold

a. For dimorphic fungus (e.g. *Histoplasma*, *Blastomyces* or *Coccidioides*)

- BacT/Alert system is not adequate.
- Recommend use of Isolator.
- If not possible to obtain Isolator, send aerobic bottle to Mycology on **Day 5**.

b. Subculture of BacT/Alert bottles

1) Mark the top of the aerobic bottle with a yellow dot indicating "blind sub to IMA".

2) On Day 5, subculture for dimorphic fungus or mold ... Inoculate 0.1 ml each to IMA (x2) and send plates and the bottle to the Mycology lab.

c. Isolators - refer to Isolator protocol.

C. Isolation of Mycobacteria

Recommend Isolator.

D. *Leptospira*

Culture is available only through consultation with Lab Director. (New York State Department of Health)

If blood specimen is needed, it must be collected in a green-top vacutainer tube (with heparin).

E. Catheter-related sepsis

Recommend use of pediatric (or adult) Isolators; one drawn through a peripheral vein, one through the catheter (Hickman, Broviac).

F. Other requests ... consult with supervisor.

IV. Computer Reports and Documentation of Culture Work-up

A. Culture workup

1. Media field:

   a. Observations (colony morphology) of bacteria/yeast growth is recorded under BAPA, MACA, CHOCA, etc.

   b. All tests that are used for identification are listed (API, Vitek, indole, ox, etc.)

   c. All results of the above tests are recorded (API profile #, Vitek ID's with low percentages, serological grouping results)

2. Isolate field

   a. Many organisms can be reflexed from the media field to the isolate field. (Be sure to hide the original bottle Gram stain result by changing the isolate number to a letter. **This will prevent a Vitek result from overwriting the original Gram stain result. Do not delete the original bottle Gram stain result.**)

   b. Be certain to have the correct isolate number when sending organisms to Vitek.

   c. View all Vitek susceptibilities.

1) Use F5 or F6 to look for suppressed or missing drugs (Ex.: ?ESBL/KPC, tigecycline, linezolid).

3. Test field

a. All changes or additions to the isolate and/or test field MUST HAVE A STATUS UPDATE.

4. Discrepancies

a. Bring to the attention of a supervisor

b. Enter the "discrep" organism in the isolate field as a letter. Enter appropriate isolate comment, indicating that slide was reviewed.

B. Finalizing cultures

1. Positive cultures

a. At the completion of identification, susceptibilities (if appropriate), and status of mate bottle (when only one bottle of set is positive), change the report from interim to final.

b. View all reports for completeness and accuracy.

c. Check HH mate bottle before finalizing.

2. Negative cultures

a. The negative list from Observa is compared to the negative worklist in the LIS.

b. Cultures are batch verified as "No Growth" and given a final status.

c. List of negative mate bottle of positive cultures (remaining on the Observa list) is given to the positive blood bench technologist.

V. Telephone Reporting of Results

A. When:

a. as soon as organisms have been seen on Gram stain and the Gram stain results have been reviewed by a checker.

b. if no organisms are seen on Gram stain, yet organism has been confirmed by repeat subculture.

c. if there is a major discrepancy between the initial report and a subsequent report.

d. N.B. Additional bottles on a patient collected on the same day, all having the same Gram stain morphology do not need to be called. An additional culture collected from the patient on a different day, regardless of Gram stain morphology, does need to be called.

B. Procedure:

1. ED **(including OBS2/2-1800)**

   Telephone tree is posted at BacT/Alert bench.

2. SMH In-patients

   Telephone the patient location. Ask for a physician or a nurse involved in the patient's care. If neither is available give the report to another member of the nursing staff.

3. MCH Patients

   Utilize call list found in the results field. Hit F9 and choose "C".

4. Clinics, Private Ambulatory, and Health Centers - notify the physician listed on the call list.

5. Discharged patients

   a. If deceased, telephone report is not needed. Enter report in LIS.

   b. For other discharged patients, use **LIS or E-Record** to obtain the name of the **ordering** physician.

BLOOD CULTURE PROCEDURE MANUAL

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