

Clinical Laboratories Procedure CULTURE FOR BIOTERRORISM AGENT			Effective: 10/17/2001
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Procedure No.	Supercedes:	Implementation Date:	Number of Pages:

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

1.0 PRINCIPLE

The clinical laboratory may be the first site at which a bio-threat (BT) agent may be detected. The laboratory must be prepared to culture appropriate specimens for suspected agents, to recognize potential BT agents if they are isolated in culture (whether suspected or not), and to properly notify the Level B lab (Santa Clara County) and package the isolates for transfer to the County lab.

2.0 SPECIMENS

2.1 Collection and Transport: Specimens from a suspected BT-infected patient for culture in the laboratory may consist of any of the following (see attached sheets for collection information), and transported as for standard processing:

Bacillus anthracis	Blood cultures, EDTA blood tube, sputum or other respiratory secretions, pleural fluid, CSF, cutaneous lesion swabs, cutaneous lesion punch biopsies or other tissue biopsies, stool or rectal swab. Serum.
Yersinia pestis	Blood cultures, EDTA blood tube, transtracheal wash (sputum may be examined but not advised for contamination with normal throat flora), CSF, tissue/bubo aspirate, tissue biopsy, skin scraping.
Clostridium botulinum	Stool (>25 g), gastric aspirate. Serum.
Francisella tularensis	Blood cultures, sputum, CSF, lymph node biopsy or aspirate. Serum.
Smallpox	Skin scraping of lesion.
Hemorrhagic virus	Blood cultures, stool, heparin tube of blood, nasopharyngeal or throat swab, urine. Serum.
Coxiella burneti	Blood cultures, tissue, body fluids, others, including cell cultures. Serum.
Alphaviruse	Blood cultures, EDTA blood tube and maintain at 4°C for storage, tissue, body fluids, others, including cell cultures. Serum.

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- 2.2 Specimen Accessioning: Check all specimens in as the appropriate routine culture for the type of specimen, but add in SDES “R/O <write name of agent>” as requested by physician.
- 2.3 Handle all specimens carefully. Wear your lab coat and gloves.

3.0 MEDIA AND SUPPLIES

- 3.1 5% Sheep blood agar, MacConkey agar, Chocolate agar, PEA agar (refrigerator), Eugon broth, Gram stain
- 3.2 Household bleach
- 3.3 10% formalin

4.0 EQUIPMENT

Standard microbiology equipment

5.0 QUALITY CONTROL

Media and Gram stain are quality controlled per our standard protocols. Hematology lab performs quality control of Wright-Giemsa stain.

6.0 PROCEDURE

- 6.1 Call Infection Control beeper and Dr. Baron or designated Director for each R/O Bioterrorism agent. One of them will coordinate sending specimens, refrigerated or in formalin, to the CDC and sending the pleural fluid to Histopath.

- 6.2 Any specimens with the following requests will NOT be processed in our lab:

R/O Botulism
R/O Tularemia
R/O Smallpox
R/O Viral hemorrhagic Fever

Specimens submitted with these requests will be held **IN THE REFRIGERATOR** until they can be transferred to Santa Clara County Lab. Notify a supervisor or lab director if you receive such specimens.

- 6.3 Specimens for R/O Anthrax and R/O Plague will be processed in our lab.

Set up the following media. After inoculating and streaking plates, place pink sticker “Suspect BT” on the back of each plate, tape the plates up, and incubate in air incubator. Also place a sticker onto any broths.

Save ALL R/O BT specimens in double-biohazard bags and place in BT bucket in specimen refrigerator.

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R/O Anthrax	
Blood cultures	1. Incubate bottles on BacT-Alert per standard protocol.
Blood EDTA tube	1. Use a disinfectant-soaked gauze, completely covering the cap, and carefully remove the rubber stopper from the tube. Aspirate small amount of blood with plastic pipette (not glass), and spread a thin circle the size of a dime on the slide (thin enough to read through). Recap the tube, place it into a clean 50 cc Falcon centrifuge tube, cap tightly, and save as for all BT specimens. Allow to dry in the hood, fix in 10% formalin for 10 min, allow to dry on the slide warmer, and Gram stain. Call technologist to examine the slide STAT.
Sputum and other respiratory secretions	1. Use a swab to inoculate SBA, Mac, CA, and Eugon broth. Streak for isolation. 2. Use a swab to prepare a smear for staining. Air dry slide, then allow to sit in 10% formalin (Coplin jar) for 10 minutes to kill spores. Then dry (on heat block) and Gram stain. Do not use results to reject specimen but include the standard comment if the Gram stain suggests oropharyngeal contamination.
Pleural fluid	1. Use a swab to inoculate SBA, Mac, CA, and Eugon broth. Streak for isolation. 2. For smear, place a heaped drop on slide (do not spread out) and allow to air dry. Fix in 10% formalin for 10 minutes, dry on heat block, and Gram stain. 3. Half of the remaining fluid will be sent to CDC for PCR. 4. Send other half of fluid to Histopathology laboratory for preparation of a sediment cell block (hold in Micro lab for Supervisor or Director to handle; contact persons in Histopath laboratory include Jim Hartinger 5-5381 and Ellen McCarthy 5-5181). Cell block will be sent to CDC for immunohistochemistry.
CSF	1. Do not cytocentrifuge, but use regular centrifuge if enough specimen is available. Discard supernatant into bleach solution. Vortex sediment in hood, and inoculate SBA, Mac, CA, and Eugon broth. 2. For smear, place a heaped drop on slide (do not spread out) and allow to air dry. Fix in 10% formalin for 10 minutes, dry on heat block, and Gram stain.
Cutaneous lesion swabs	1. Inoculate SBA, Mac, CM, CA, and Eugon with one swab. Streak for isolation. 2. Prepare Gram stain with other swab. Air dry slide, then allow to sit in 10% formalin (Coplin jar) for 10 minutes to kill spores. Then dry (on heat block) and Gram stain.
Tissue biopsies	1. For the first biopsy (not sent to the lab in formalin), call a supervisor. Will need to grind up in a little bit of Eugon. Inoculate SBA, CM, Mac, CA, and Eugon with one drop per plate. Streak for isolation. Prepare a thin Gram stain. Air dry slide, then allow to sit in 10% formalin (Coplin jar) for 10 minutes to kill spores. Then dry (on heat block) and Gram stain. 2. Second biopsy (sent to the lab in formalin) will be sent to CDC for histopathology, immunohistochemical staining, and PCR.
Stool or rectal swab	1. Use a swab to inoculate SBA, Sorb Mac, Campy, HE, Mac, and PEA agar. Streak for isolation.
Serum	1. Centrifuge red or gold top tube. Pipette off the supernatant (i.e. serum) which will be sent to CDC for serology.

R/O Plague	BSL-2 laboratory
Pneumonic plague- Bronchial or transtracheal wash (Sputum may be examined but not advised for contamination with normal throat flora)	Inoculate SBA, Mac, and Eugon broth. Streak for isolation. Incubate in ambient atmosphere at 28 ⁰ C (optimal), 35 ⁰ C (slow growth) for 5 days or 7 days if the patient is on bacteriostatic antibiotic. Prepare slides for Gram & Wright-Giemsa stain. Air dry, fix in 10% formalin for 10 min, then dry on heat block for 5 min., and send one slide to Hematology lab for Wright-Giemsa staining. Gram stain the other slide and call technologist to examine stat.
Septicemic plague- Blood culture	Incubate one 2 aerobic and 1 anaerobic bottles on BacT-Alert per standard protocol. Incubate one aerobic bottle at 28 ⁰ C without shaking for culture characteristics. [See Blood Culture for <i>Y.pestis</i> Protocol]
Blood EDTA tube	Use a disinfectant-soaked gauze, completely covering the cap, and carefully remove the rubber stopper from the tube. Aspirate small amount of blood with plastic pipette (not glass), and spread a thin circle the size of a dime on two slides (thin enough to read through). Recap the tube, place it into a clean 50 cc Falcon centrifuge tube, cap tightly, and save as for all BT specimens. Allow the smear to dry in the hood, fix in 10% formalin for 10 min, allow to dry on the slide warmer, and Gram stain one slide. Call technologist to examine the slide STAT. Send another slide to Hematology lab for Wright-Giemsa staining.
Bubonic plague Aspirate of bubo	Inoculate SBA, Mac, and Eugon broth. Streak for isolation. Incubate in ambient atmosphere at 28 ⁰ C (optimal), 35 ⁰ C (slow growth) for 5-7 days. Prepare slide for Wright-Giemsa stain and Gram stain. Air dry, 10% formalin for 10 min, then dry on heat block and send one slide to Hematology lab for Wright-Giemsa staining. Gram stain the other slide and call technologist to examine stat.
Biopsied specimen from liver, spleen, bone marrow, lung	Call a supervisor. May need to grind up in a little bit of Eugon. Inoculate SBA, Mac, and Eugon broth. Streak for isolation. Incubate in ambient atmosphere at 28 ⁰ C (optimal), 35 ⁰ C (slow growth) for 5-7 days Prepare slides for Wright-Giemsa stain and Gram stain. Air dry, fix in 10% formalin for 10 min, then dry on heat block and send one slide to Hematology lab for Wright-Giemsa staining. Gram stain the other slide and call technologist to examine stat.

- 6.4 If you will use pipettes, slides, swabs, or other solid or re-usable items for processing, dilute household bleach 1:10 in a pan or bucket and soak the tools, slides, etc. for 1 hour before removing for autoclaving or to deposit into sharps container or other discard container.
- 6.5 After setting up the specimens, wipe surface down with sporicidal disinfectant (1:10 dilution of bleach). Then wipe again with regular disinfectant to remove bleach.
- 6.6 Incubate all plates and broths in air incubator at 35⁰ C for R/O Anthrax and at 28⁰C and 35⁰C for R/O plague specimens..

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9.0 LIMITATIONS

Failure to isolate a BT agent from cultures does not totally rule out infection with that agent or other agents.

10.0 REFERENCES

Center for Disease Control (CDC)

<http://www.bt.cdc.gov/>

Updated information on current outbreaks, information, links regarding bioterrorism

American Society for Microbiology (ASM)

<http://www.asmtusa.org/pcsrc/bioprep.htm>

Resources related to biological weapons control and Bioterrorism preparedness including latest CDC/ASM/APHL Level A Laboratory protocols

Infectious Diseases Society of America

<http://www.idsociety.org/>

Journal of the American Medical Association

<http://jama.ama-assn.org/>

Journal issues focusing on bioterrorism agents with an excellent series of articles on the medical presentation and management of Anthrax, Smallpox, Plague, Botulism and Tuleremia (Full articles available as PDF downloads)

Modifications

DATE	DESCRIPTION	SIGNATURE
11/15/01	Expansion of R/O Anthrax Procedure Notes and References as per latest ASM/CDC guidelines.	
01/08/02	R/O Plague Procedure Notes and references as per latest ASM/CDC/APHL guidelines	
08/21/02	Changed specimen holding temp from RT to Refrigerator	