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PRACTICAL APPROACH TO BIOSAFETY AND BIOTERRORISM IN THE ROUTINE CLINICAL MICROBIOLOGY LABORATORY

INTRODUCTION

The previous and recent events worldwide have created a heightened awareness and concern regarding the potential of a bioterrorist attack. Because such an attack could be overt (announced/broadcast) or covert, the microbiology laboratory may play an important role in the initial identification and control of spread of potentially infectious agents. Although many biological agents could be used as weapons of bioterrorism, the following are considered the most likely:

1. *Bacillus anthracis* (Anthrax)
2. *Francisella tularensis* (Tularemia)
3. *Yersinia pestis* (Plague)
4. *Brucella* spp. (Brucellosis)
5. Botulism toxin (*C. botulinum*)
6. Variola virus (Smallpox)

There are 3 possible scenarios which may occur and may involve the Microbiology Department directly or indirectly. The following will deal with each scenario as well as the appropriate handling, microbiologic work-up and reporting of the above pathogens.

SCENARIOS / SPECIMEN PROCESSING

Scenario I: Suspicious letter/package

A person opens a letter/package containing a suspicious powder/substance and contacts the Microbiology Department asking how to proceed.

1. The person should be instructed to proceed as follows:
   (i) Place the envelope or package in a plastic bag. If a plastic bag is not available, or powder has spilled out, cover the area, and do not further disturb it. The package should be kept for the emergency services team, and not disturbed. **Do not send the package to the microbiology lab**.
   (ii) If the scene occurs in the hospital, activate the hospital's emergency response procedure for a biohazard threat (at Mount Sinai Hospital, call ext. 5133), then

notify the area manager/supervisor. If the scene occurs outside the hospital, call 911.

(iii) Ensure that any person who has touched the envelope/package wash their hands and face.

(iv) Identify anyone who is in the immediate area, and ensure that they remain in the area until the emergency response team arrives.

(v) Keep all other people out of the area until the emergency response team arrives.

2. The laboratory personnel receiving this call should page infection control and the microbiologist on call.

NB: Please also refer to the Hospital Emergency Manual.

Scenario II: Specimen collection for suspected biological agents

Clinician/Physician telephones asking what specimens to be sent to the Microbiology Department for work-up of a patient with a suspected clinical diagnosis involving one of the potential bioterrorist agents listed above.

1. The physician should be referred to the medical microbiologist on call to discuss the case. The medical microbiologist will notify infection control.

2. Appropriate specimens (as listed in Specimens To Be Collected For Detection of Suspect Agents of bioterrorism) should be collected and sent immediately to the Microbiology Department with completed requisitions noting the clinical diagnosis and suspected agent(s).

   Note: Nasal swabs are not an appropriate specimen. They are useful in outbreak investigations to assess the extent and degree of risk, and improve our ability to manage exposures in the future. Persons with a significant exposure to confirmed anthrax should receive prophylaxis whether they have a positive nasal swab or not.
   Nasal swabs in unexposed persons, or those exposed to a powder which is NOT confirmed to be anthrax, are not helpful. For ill persons, blood cultures and lesion specimens are diagnostic, and nasal swabs are not recommended.
   Nasal swabs received in the laboratory will be stored, and reported as "Specimen held but not processed. Nasal swabs are useful only for epidemiologic investigation. This specimen will be processed at the request of public health. Please call the medical microbiologist on call for information."

3. The physician should contact the Infectious Diseases Service requesting an urgent consult.

4. When specimens arrive in the laboratory, they should be processed and worked up as outlined below. All microbiology staff handling or processing such specimens should do so following standard Level II biological safety guidelines. All specimen handling and processing should take place in a Level II biological safety cabinet.

Scenario III: Specimen processing and presumptive identification of suspect biological agents

If, based on the Gram stain and/or culture results, one of the above noted biological agents is suspected, regardless of whether it was suspected clinically, appropriate work-up, identification, and reporting should proceed.

For specimen processing and presumptive identification of suspect biological agents, follow the instructions in **Suspect Risk Group 3_4 Biosafety Manual (Policy # MI_RG34)**

Specimens To Be Collected For Detection of Suspect Agents of bioterrorism

Suspected Agent	Site / Route of Infection	Specimens[1]
Bacteria:		
• *Bacillus anthracis* (Anthrax)	• Inhalation / Pneumonic • Cutaneous • Gastrointestinal • Exposed Individual	• Blood culture, Sputum, ± CSF • Swab / aspirate of cutaneous lesion or vesicular fluid, Blood culture • Blood culture, Stool • Nasal Swab *
• *Francisella tularensis* (Tularemia)	• Pneumonic • Cutaneous	• Blood culture, Sputum, Bronchial washings • Lymph nodes, Wound swab / aspirate
• *Brucella* spp. (Brucellosis)	• Systemic	• Blood culture, Bone marrow, ± Spleen, ± Liver, Abscess material • Acute & Convalescent serum (21 to 28 days apart) (Red top tube, 10 ml)
• *Yersinia pestis* (Plague)	• Pneumonic • Systemic	• Blood culture, Sputum, Bronchial washings • Above ± Spleen, ± Liver, ± Bubo aspirate
Toxin:		
• Botulism toxin (Botulism)	• Systemic / neurologic	• 10 mL of Serum (Red top tube, 2 tubes of 10 mL blood) • Vomitus / Gastric contents, stool, tissue or Wound anaerobic swab • Food Samples
Virus:		
• Variola virus (Smallpox)[2]	• Cutaneous lesions	• Vesicular fluid, Lesion biopsy, Lesion scabs/ scrapings

[1]All specimens can be transported to the lab at room temperature EXCEPT:
a) Specimens for Variola virus should be kept at 4°C (refrigerated) or frozen at -20°C or lower;
b) Specimens for botulism toxin should be kept at 4°C (refrigerated)

* Nasal swab is useful only for outbreak investigation and will be processed only if ordered by the Public Health department.

[2]The MOH must be immediately notified of any case of suspect smallpox. Prior to sending any specimen to PHL, one of the Medical Microbiologists must be notified. All specimens for suspect smallpox will be forwarded to NML in Winnipeg. This is a level IV agent!

Processing of Specimens for Detection of Suspect Agents of Bioterrorism

Suspected Agent	Specimen	Media[1]	Incubation[2]
B. anthracis	• Blood • Sputum • Stool • Cutaneous/ nasal swab	BacT/Alert Bottles BA, CHOC, FAB Routine & CNA BA, CHOC, FAB	Virtuo x 5 days[3] O_2, 35°C x 48 hrs O_2, 35°C x 48 hrs
F. tularensis	• Sputum, Bronchial washings, wounds, lymph nodes	BA, MAC, CHOC, BCYE	CO_2, 35°C x 72 hrs
Brucella spp.	• Blood, Bone marrow • Tissue, Wounds • Serum	BacT/Alert Bottles BA, MAC, CHOC Forward to Central Public Health Lab for testing at room temperature	Virtuo x 21 days[3] 5% CO_2, 35°C x 7 days
Y. pestis	• Blood • Sputum, Bronchial washings, Tissues	BacT/Alert Bottles BA, MAC, CHOC	Virtuo x 5 days[3] O_2, 28°C x 48 hrs
Botulism toxin (*C. botulinum*)	• All	Forward to Central Public Health Lab for testing on wet ice	
Variola virus	• All	Forward to Central Public Health Lab for testing on wet or dry ice	

[1]BA = 5% Sheeps blood agar; MAC = MacConkey agar; CHOC = Chocolate Agar; BCYE = Buffered Charcoal Yeast Extract Agar; BRUC = Fastidious Anaerobic Agar, FAB=Fastidious anaerobic broth
[2]Examine plates at 18-24 hrs, 48 hrs and daily thereafter for suspicious colonies as noted below.
[3]Do not perform blind subcultures; If blood culture becomes positive, perform gram stain and subculture onto BA, CHOC, MAC. Add additional media as indicating by the gram stain.

Profile of Risk Group 3 Organism Cultures

B. anthracis

Gram Stain:

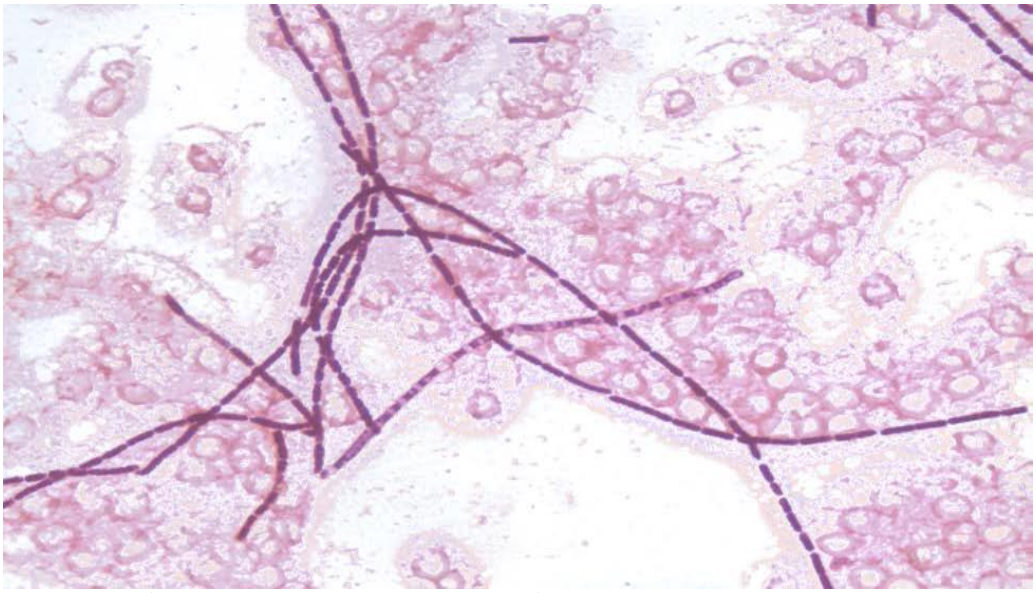


Photo courtesy of Dr. James Rudrick, Michigan Department of Community Health
https://www.asm.org/images/PSAB/LRN/Anthrax%20LRN%20091217.pdf

Direct smear from clinical samples:

- large (1.0 to 1.5 μm by 3 to 5 μm) encapsulated gram positive bacilli in short chains.
- Gram stain can demonstrate clear zones (capsule) around rods.
- Spores usually not present in clinical specimens unless exposed to atmospheric O_2.

Smears from sheep blood agar or other routine nutrient medium

- Large Gram positive bacilli in long chains, usually non-encapsulated.
- Oval, central to subterminal spores: 1 x 1.5 μ with no significant swelling of cell.

Culture:

Photo courtesy of APHL
https://www.asm.org/images/PSAB/LRN/Anthrax%20LRN%20091217.pdf

B. anthracis grows rapidly; heavily inoculated areas may show growth on a blood agar plate within 6-8 h and individual colonies may be detected within 12-15 h. This trait can be used to isolate *B. anthracis* from mixed cultures containing slower-growing organisms.

On Sheep Blood Agar (SBA) - Nonhemolytic, flat or slightly convex colonies with ground-glass appearance; tenacious consistency (Hemolysis on SBA excludes *B. anthracis*). Often have comma-shaped protrusions from colony edge ("Medusa head" colonies).
If isolate is non-hemolytic, perform motility test using motility test media (*B. anthracis* is non-motile).
B. anthracis will not grow on McConkey (MAC) agar with crystal violet. Since the MAC plate we use is without crystal violet, this characteristic is not useful; this is why we do not include MAC as a media for primary isolation to avoid confusion.

Presumptive identification:
Presumptive identification of *B. anthracis* is based on identification of large gram positive bacilli that are **nonhemolytic** on SBA and **non-motile**. When a suspect species is identified, follow WHAT TO DO IF A RISK GROUP 3 ORGANISM IS SUSPECTED. Otherwise, report as "Bacillus species isolated" (from sterile sites) or as part of "Commensal flora" (from non-sterile sites such as wounds).

If a presumptive *B. anthracis* colony is identified and suspected as a bioterrorist threat agent**:** Preserve original specimens pursuant to a potential criminal investigation.

F. tularensis

Gram Stain:

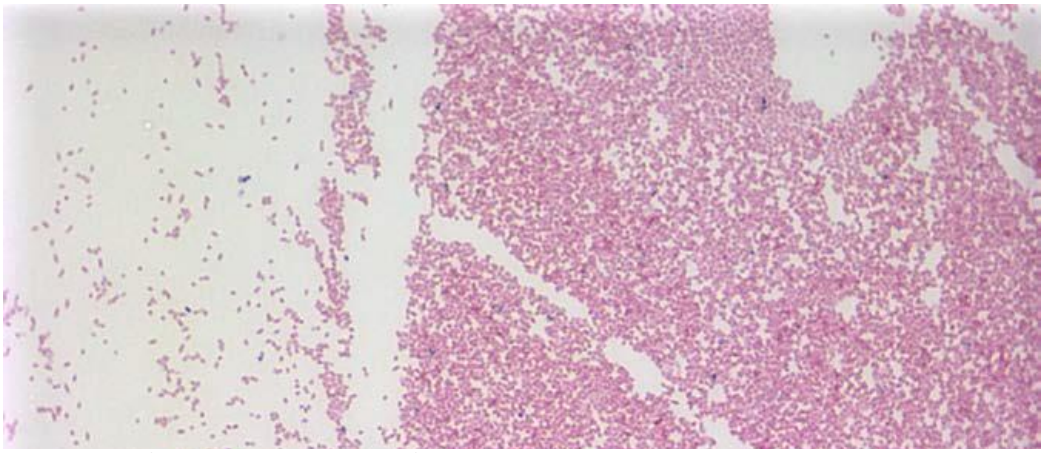


Photo courtesy of Cheryl Gauthier, MA Dept. of Public Health
https://www.asm.org/images/PSAB/LRN/Tularemia316.pdf

Tiny (0.2 to 0.5 μm by 0.7 to 1.0 μm), poorly staining pleomorphic gram negative bacilli / coccobacilli.

Culture:

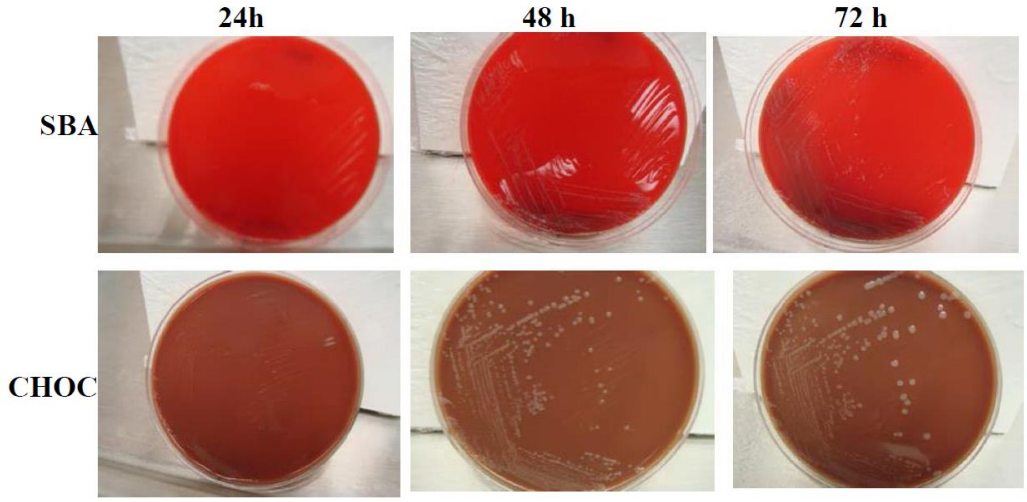


Photo courtesy of: MAJ Todd Kijek, USAMRIID
https://www.asm.org/images/PSAB/LRN/Tularemia316.pdf

SBA - Non-hemolytic, gray-white colonies, 1-2 mm after 48 hrs MAC - No growth

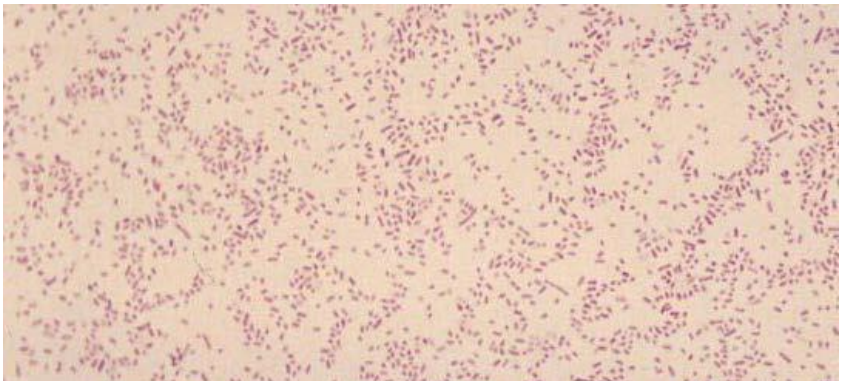
Francisella are also catalase positive, oxidase negative and urease negative which grow on BA & BCYE but not MAC.
DO NOT perform any tests in any circumstance for small/tiny gram negative bacilli.

When tiny gram negative bacilli/coccobacilli are identified, follow WHAT TO DO IF A RISK GROUP 3 ORGANISM IS SUSPECTED.

Report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

***Brucella* spp**

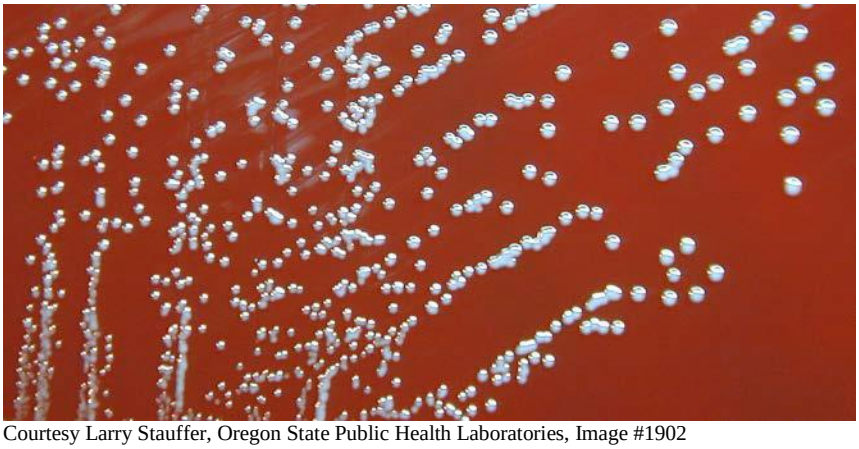
Gram Stain:



https://www.asm.org/images/PSAB/LRN/Brucella316.pdf

Tiny (0.5 to 0.7 μm by 0.6 to 1.5 μm), faintly staining, gram negative coccobacilli

Culture:



Courtesy Larry Stauffer, Oregon State Public Health Laboratories, Image #1902
https://www.asm.org/images/PSAB/LRN/Brucella316.pdf

SBA - Small (0.5 to 1.0 mm) glistening, non-hemolytic, non-pigmented colonies after 2 to 3 days incubation
MAC - Some strains may grow slowly

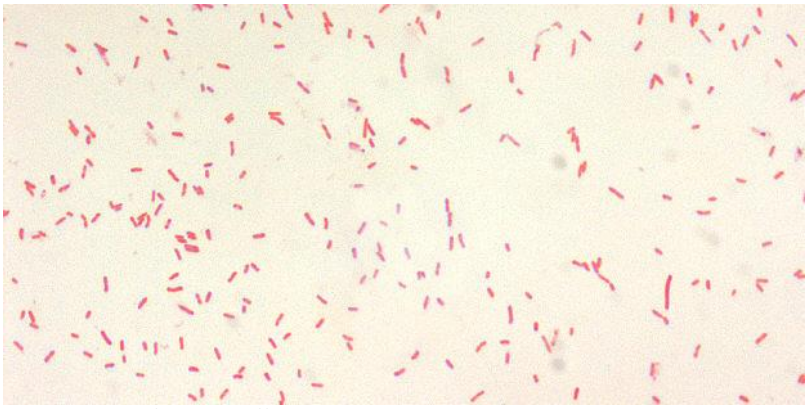
Brucella spp. are also are oxidase positive and urea hydrolysis positive.
DO NOT perform any tests in any circumstance for small/tiny gram negative bacilli.

When tiny gram negative bacilli/coccobacilli are identified, follow WHAT TO DO IF A RISK GROUP 3 ORGANISM IS SUSPECTED.

Report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

Y. pestis

Gram Stain:



CDC/ Courtesy of Larry Stauffer, Oregon State Public Health Laboratory
https://phil.cdc.gov/details_linked.aspx?pid=1915

Gram negative bacilli (1.0 by 0.5 μm) that may exhibit bipolar staining

Culture:

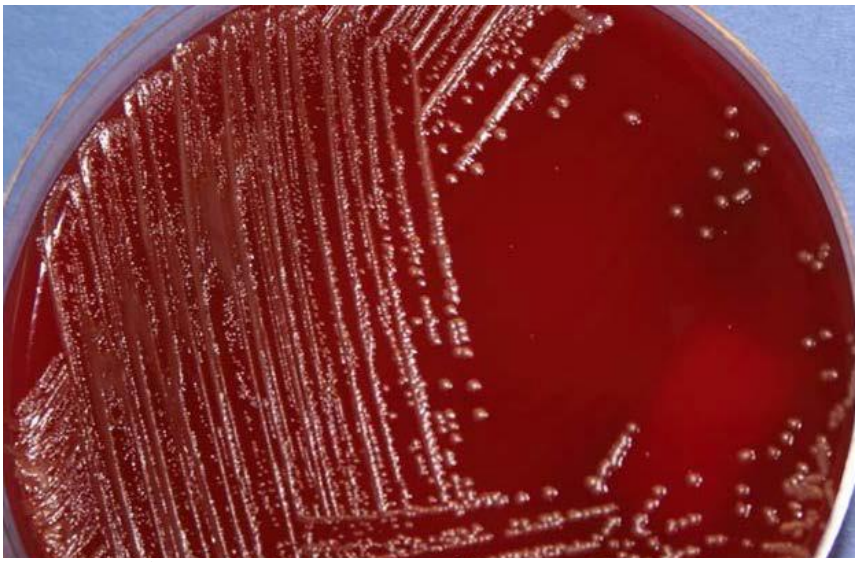


Photo courtesy of APHL
https://www.asm.org/images/PSAB/LRN/Ypestis316.pdf

SBA - gray-white to slightly yellow opaque colonies after 48 hrs incubation; Beyond 48 to 72 hrs incubation, colonies develop fried egg appearance. Little or no hemolysis.
MAC - small, lactose negative colonies after 24 hrs incubation.

When slow growing gram negative bacilli as per growth characteristics described, follow WHAT TO DO IF A RISK GROUP 3 ORGANISM IS SUSPECTED.

Report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

REPORTING

If any of the above organisms is presumptively identified, proceed as follows:

1. Notify the medical microbiologist on call immediately.
2. Prepare a subculture of the organism on Trypticase soy agar (TSA) for shipping to the Central Public Health Lab.
3. Notify the Central Public Health Lab [During Business Hours: Dr. Frances Jamieson (416) 235-5712 or Dr. Margaret Fearon (416) 235-5725; After Hours: Call the Duty Officer (416) 605-3113] that an isolate will be sent for further identification.
4. Do not report the presumptive result in the LIS until further instructions from the microbiologist.
5. If a presumptive *B. anthracis* colony is identified and suspected as a bioterrorist threat agent**:** Preserve original specimens pursuant to a potential criminal investigation.

6. The medical microbiologist will:

   I. Contact the treating physician to review the case.
   II. Notify the senior hospital administrator on call.
   III. Notify the Infection Control Department.
   IV. Notify Toronto Public Health:
   - During business hours: Tel: (416) 392-7411
   - After hours: Tel: (416) 690-2142

PACKAGING AND TRANSPORTING PROTOCOL

- Suspected isolates will be packaged for transport to PHL according to the Transportation of Dangerous Goods regulation. Staff certified for transportation of dangerous goods will do the packaging.
- Inform Microbiologist to arrange for special courier (either special courier from PHL or lab personnel to drive to PHL)

REFERENCES

1. Klietmann WF and Ruoff KL. 2001. Bioterrorism: Implications for the Clinical Microbiologist. Clin Microbiol Rev 14:364-381.

2. Murray PR, Baron EJ, et al. 1999. Manual of Clinical Microbiology. 7th Ed. ASM Press, Washington, DC.

3. Basrur SV. Bioterrorism (Letter, October 10, 2001) Medical Officer of Health, Toronto Public Health.

4. CDC Guidelines for State Health Departments (Revised October 14, 2001)

5. CDC Basic protocol for the presumptive identification of Bacillus anthracis

6. Guideline and Fact sheet form Ontario Ministry of Health October 15, 2001

Record of Edited Revisions

Manual Section Name: Bioterrorism Procedure Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	March 01 2002	Dr. T. Mazzulli
Annual Review	May 12 2003	Dr. T. Mazzulli
Annual Review	May 26 2004	Dr. T. Mazzulli
Annual Review	May 12 2005	Dr. T. Mazzulli
Annual Review	July 23 2006	Dr. T. Mazzulli
Annual Review	August 13 2007	Dr. T. Mazzulli
Annual Review	August 17 2008	Dr. T. Mazzulli
Annual Review	August 20, 2009	Dr. T. Mazzulli
Annual Review	August 20, 2010	Dr. T. Mazzulli
Annual Review	May 31, 2011	Dr. T. Mazzulli
Annual Review	May 31, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
Pathology & Laboratory Medicine - Emergency Preparedness Plan D0004273.doc Link Added Annual Review	September 17, 2014	Dr. T. Mazzulli
Annual Review	April 14, 2015	Dr. T. Mazzulli
Annual Review Update MSH logo in header	April 02, 2016	Dr. T. Mazzulli
Annual Review Update MSH logo in header	April 07, 2017	Dr. T. Mazzulli
Annual Review Addition of Biosafety procedures: How to Identify and what to do when potential RG3 organism are suspected. Addition of Flowchart of suspect RG3 organisms. Addition of gram and culture images. Removal of instruction to perform any testing including oxidase, catalase, urease on suspect RG3 organisms.	April 04, 3018	Dr. T. Mazzulli
Minor format change	September 14, 2018	Dr. T. Mazzulli
Annual Review	October 20, 2019	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Minor formatting change	April 11, 2021	Jessica Bourke
Added "For specimen processing and presumptive identification of suspect biological agents, follow the instructions in Suspect Risk Group 3_4 Biosafety Manual (Policy # MI_RG34)" on page 4	March 15, 2022	Oliver Li