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BIOTERRORISM PROCEDURE MANUAL

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Section: Bioterrorism Procedure Manual	Subject Title: Laboratory Manual for Handling Bioterrorism Samples	
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**PRACTICAL APPROACH TO BIOTERRORISM IN THE ROUTINE
CLINICAL MICROBIOLOGY LABORATORY**

I. INTRODUCTION

The recent events in the USA 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.

II. 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 do not have a plastic bag, 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.**

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- (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 have 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 Table 1) 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."

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

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 possible 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 as outlined below.

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Table 1. Specimens to be Collected for Detection of Possible Agents of Bioterrorism

Suspected Agent	Site / Route of Infection	Specimens ¹
Bacteria:		
<ul style="list-style-type: none"> <i>Bacillus anthracis</i> (Anthrax) 	<ul style="list-style-type: none"> Inhalation / Pneumonic Cutaneous 	<ul style="list-style-type: none"> Blood culture, Sputum, ± CSF Swab / aspirate of cutaneous lesion or vesicular fluid, Blood culture Blood culture, Stool Nasal Swab *
<ul style="list-style-type: none"> <i>Francisella tularensis</i> (Tularemia) 	<ul style="list-style-type: none"> Gastrointestinal Exposed Individual Pneumonic Cutaneous 	<ul style="list-style-type: none"> Blood culture, Sputum, Bronchial washings Lymph nodes, Wound swab / aspirate
<ul style="list-style-type: none"> <i>Brucella</i> spp. (Brucellosis) 	<ul style="list-style-type: none"> Systemic 	<ul style="list-style-type: none"> Blood culture, Bone marrow, ± Spleen, ± Liver, Abscess material Acute & Convalescent serum (21 to 28 days apart) (Red top tube, 10 ml)
<ul style="list-style-type: none"> <i>Yersinia pestis</i> (Plague) 	<ul style="list-style-type: none"> Pneumonic Systemic 	<ul style="list-style-type: none"> Blood culture, Sputum, Bronchial washings Above ± Spleen, ± Liver, ± Bubo aspirate
Toxin:		
<ul style="list-style-type: none"> Botulism toxin (Botulism) 	<ul style="list-style-type: none"> Systemic / neurologic 	<ul style="list-style-type: none"> Serum (Red top tube, 10 ml) Vomit / Gastric contents, stool, tissue or Wound anaerobic swab Food Samples
Virus:		
<ul style="list-style-type: none"> Variola virus (Smallpox) E 	<ul style="list-style-type: none"> Cutaneous lesions 	<ul style="list-style-type: none"> Vesicular fluid, Lesion biopsy, Lesion scabs/ scrapings

¹All specimens can be transported to the lab at room temperature EXCEPT:

- Specimens for Variola virus should be kept at 4°C (refrigerated) or frozen at -20°C or lower;
- 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.

EThe 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 a a level IV agent!

Table 2. Processing of Specimens for Detection of Possible Agents of Bioterrorism

Suspected Agent	Specimen	Media ¹	Incubation ²
<i>B. anthracis</i>	• Blood	BacT/Alert Bottles	BacT-Alert x 5 days ³
	• Sputum	BA, CHOC, FAB	O ₂ , 35°C x 48 hrs
	• Stool	Routine & CNA	
	• Cutaneous/ nasal swab	BA, CHOC, FAB	O ₂ , 35°C x 48 hrs
<i>F. tularensis</i>	• Sputum, Bronchial washings, wounds, lymph nodes	BA, MAC, CHOC, BCYE	CO ₂ , 35°C x 72 hrs
<i>Brucella</i> spp.	• Blood, Bone marrow	BacT/Alert Bottles	BacT-Alert x 21 days ³ 5% CO ₂ , 35°C x 7 days
	• Tissue, Wounds	BA, MAC, CHOC	
	• Serum	Forward to Central Public Health Lab for testing at room temperature	
<i>Y. pestis</i>	• Blood	BacT/Alert Bottles	BacT-Alert x 5 days ³
	• Sputum, Bronchial washings, Tissues	BA, MAC, CHOC	O ₂ , 28°C x 48 hrs
Botulism toxin (<i>C. botulinum</i>)	• 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	

¹BA = 5% Sheeps blood agar; MAC = MacConkey agar; CHOC = Chocolate Agar; BCYE = Buffered Charcoal Yeast Extract Agar; BRUC = Fastidious Anaerobic Agar, FAB=Fastidious anaerobic broth

²Examine plates at 18-24 hrs, 48 hrs and daily thereafter for suspicious colonies as noted below.

³Do 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.

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Table 3. Work-up of cultures/isolates for Presumptive Identification of Possible Agents of Bioterrorism
(**NB:** Process all specimens and cultures in a Level II Biological Safety Cabinet)

1. ***B. anthracis*:**

Gram Stain:

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 μm with no significant swelling of cell.

Culture :

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).

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.

If isolate is non-hemolytic, perform motility test using motility test media (*B. anthracis* is non-motile) and subculture to Trypticase soy agar slant for shipment to PHL if necessary.

Presumptive identification of *B. anthracis* is based on identification of large gram positive bacilli that are **nonhemolytic** on SBA and **non-motile**. If presumptive diagnosis of *B. anthracis*, proceed as outlined below under "Reporting". Otherwise, report as

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"Bacillus species isolated" (from sterile sites) or as part of "Commensal flora" (from non-sterile sites such as wounds).

2. ***F. tularensis*:**

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

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

If isolate meets criteria above, in a Level II Biological Safety Cabinet perform catalase, oxidase and urease.

Presumptive identification of *F. tularensis* is based on identification of tiny, poorly staining, pleomorphic gram-negative bacilli / coccobacilli that are catalase positive, oxidase negative and urease negative which grow on BA & BCYE but not MAC.

If presumptive diagnosis of *F. tularensis*, procede as outlined below under "Reporting". Otherwise, forward isolate to Central Public Health Lab for further identification and report as "Gram negative bacillus / coccobacillus isolated. Further identification to follow".

3. ***Brucella spp.*:**

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

Culture: 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

If isolate meets criteria above, in a Level II Biological Safety Cabinet perform oxidase and urea hydrolysis.

Presumptive identification of *Brucella spp.* is based on identification of faintly staining coccobacilli that are oxidase positive and urea hydrolysis positive.

If presumptive diagnosis of *Brucella spp.*, procede as outlined below under "Reporting". Otherwise, forward any isolates which cannot be further identified in the clinical laboratory to the Central Public Health Lab for further identification and report as "Gram negative coccobacillus isolated. Further identification to follow".

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4. *Y. pestis*:

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

Culture: 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.

Identify these isolates following standard laboratory procedures for gram negative bacilli including oxidase, Vitek card, API, or other tests as appropriate.

If a presumptive diagnosis of *Y. pestis*, proceed as outlined under "Reporting" below. Otherwise, report as noted elsewhere in the manual for gram negative bacilli.

III. **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

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IV. 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)

V. REFERENCES

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