DRAFT
FROM CAREC
LABORATORY SAFETY GUIDELINES
FOR
HANDLING
SUSPECTED ANTHRAX
SAMPLES
LABORATORY SAFETY GUIDELINES FOR HANDLING SUSPECTED ANTHRAX SAMPLES

All testing procedures must be performed within a certified Class II biological safety cabinet (BSC).

When handling the Bioterrorism agent, white powder the following should be implemented:
- The use of respirators which filter >90% of particles ranging from 0.5 um to 1.0 um e.g. N95 respirators from 3M.
- Safety glasses or eye shields are recommended
- Laboratory coats
- Gloves
- Cover the work surface with absorbent material that is saturated with disinfectant (1:10 bleach)
- The blower of the BSC should not be turned on until the powder is suspended in sterile water and the work area decontaminated of any spilled powder. This will avoid any dispersal of the powder.
- Ensure that the cabinet does not contain any unnecessary items that will interfere with the airflow.
- Any activities that bring hands into contact with mucosal surfaces such as eating, drinking, smoking and applying make-up are prohibited in the laboratory.
- Protective clothing must not be worn outside the work area and must be placed in separate containers or laundry bags and autoclaved before being washed or discarded. Under no circumstances must protective clothing be taken home to be laundered.
- Proper hand washing is required before leaving the laboratory.
- Anthrax vaccination is not required.

DECONTAMINATION

1. Commercially available household bleach solution containing 5.25% hypochlorite, when diluted 1:10 is effective in routine decontamination of surfaces and instruments after working with B. anthracis.

2. Contaminated items such as pipettes, disposable loops etc. should be immersed in decontamination solution until autoclaving.

3. Spills involving fresh cultures or samples known to have low concentrations of the organism should be flooded with decontamination solution and soaked for 5 minutes before clean up.

4. Spills that involve samples with high concentrations of the organism, organic matter, or occur in areas of lower than room temperature (refrigerators, freezers) should be exposed to decontamination solution for at least one hour before clean up.

5. Persons involved in the clean up of spills should wear gloves, safety glasses, and a laboratory coat or gown during the clean up process.

6. Respiratory protection should be considered for spills in which a substantial aerosolization is suspected when handling the powder.
WASTE DISPOSAL

a. All infectious waste must be placed in autoclavable pans or bags for decontamination.
b. Disinfectant solution must be placed in discard pans so as to begin the decontamination process.
c. All infectious waste must be autoclaved and / or incinerated before disposal
d. Waste should be autoclaved as close to the point of generation as possible.
e. Biological indicators must be used to monitor the effectiveness of the autoclaving process. Waste may also be autoclaved centrally (away from the lab) however, it must be packaged in leakproof bags and containers and must be properly transported to the central autoclave.
f. After autoclaving, ideally, all waste should be incinerated for complete destruction.
g. All waste handlers must be properly trained to handle infectious laboratory waste.

TRANSPORTATION OF SPECIMENS

Laboratories that do not have the facilities to perform the testing should contact CAREC for further information.

Specimens that are to be transported on land must be packaged so as to fully contain the specimen while in transit.

The triple packaging system must be used.

1. **The primary receptacle.** The specimen must be placed in a leakproof primary receptacle. If the specimen is a powder in an envelope, place this envelope in a zip lock bag.

2. **The secondary receptacle.** This is a durable secondary container that encloses and protects the primary receptacle. If the specimen is a liquid there must be sufficient absorbent material between the primary and secondary receptacles to absorb the entire contents of the primary receptacle.

3. **The outer package.** The secondary receptacle must be placed in an outer package that protects it from physical damage.

Specimens that are to be transported by air must be shipped as "Infectious substance, affecting humans" of "Infectious substance, affecting animals" and packaged in accordance with the International Air Transportation Association, Dangerous Goods Regulations.

OR

Refer to the WHO "Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens" [www.who.int/emc/biosafety.html](http://www.who.int/emc/biosafety.html)
LABORATORY PROCEDURES FOR THE IDENTIFICATION OF *BACILLUS ANTHRACIS*

**Specimen Type:**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Preparation</th>
<th>Inoculation</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>White powder</td>
<td>Suspend a small amount in approximately 1ml of sterile distilled water.</td>
<td>Streak out one drop of suspension on each of:</td>
<td>$O_2$ 35³C - 37³C x 18-24 hrs</td>
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<tr>
<td>(collected in blood culture bottles)</td>
<td>- Cap tube.</td>
<td>-Sheep blood agar (SBA)</td>
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<td></td>
<td>-Vortex.</td>
<td>-Chocolate agar (CHOC)</td>
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<td></td>
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<td>-MacConkey agar (MAC)</td>
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<tr>
<td></td>
<td></td>
<td>Innoculate 1 ml of suspension in 10ml</td>
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<tr>
<td></td>
<td></td>
<td>Enrichment Broth*</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>Sub positives on SBA, CHOC &amp; MAC.</td>
<td>As above</td>
<td></td>
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<tr>
<td>(collected in blood culture bottles)</td>
<td>-Gram stain.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>SBA, CHOC , MAC -Gram stain</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td>Swab/aspire of cutaneous lesion or vesicular fluid</td>
<td>Centrifuge (1500xg for 15min) Remove supernatant. Gram and culture deposit</td>
<td>-SBA, CHOC - Gram stain</td>
<td>As above</td>
</tr>
<tr>
<td>(collect in ordinary bacteriology transport medium )</td>
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<td></td>
<td></td>
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<tr>
<td>Blood</td>
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<tr>
<td>CSF</td>
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<td>Stool</td>
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<td>Stool</td>
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**Transportation:**

Transport all specimens to the laboratory at room temperature.

**Specimen Processing:**

All testing procedures must be performed within a certified Class II biological safety cabinet (BSC). (Refer to the laboratory safety guidelines above)
* Subculture enrichment broth to SBA, CHOC, MAC. Incubate at 35°C-37°C for 18 – 24 hrs.

Some reference laboratories are not using enrichment broth, however we recommend using an enrichment culture until we seek further clarification

**Examination of plates:**

Examine plates at 18-24 hours.

**Colony characteristics of B. anthracis:** On SBA at 18- 24 hours, colonies are:
- 2-5mm in diameter
- Flat or slightly convex
- Irregularly round with a wavy border
- Have a ground glass appearance
- Often have comma shaped projections from the colony edge producing the "Medusa head" colony
- Colonies have a tenacious consistency i.e. when teased with a loop the growth will stand up like beaten egg white
- Colonies are non –hemolytic
- Colonies grow rapidly – individual colonies may be detected within 12-15 hours.

**Further tests:**

**Gram stain:**
- B. anthracis is a large gram positive rod (1-1.5 x 3-5 um) that forms oval, central to subterminal spores (1 x 1.5 um) on SBA that do not cause significant swelling of the cell.
- Spores are not present in clinical samples unless exposed to atmospheric levels of CO₂
- Vegetative cells seen on Gram stain of blood and impression smears are in short chains of 2-4 cells that are encapsulated
- Gram stain from growth on SBA occur as long chains of bacilli and are not encapsulated

**Motility:** B. anthracis is nonmotile. However a few non-pathogenic Bacillus spp are non-motile

**Presumptive Identification key for B anthracis :**
- Typical colony as described above
- From clinical samples - encapsulated gram positive rods
- Gram positive broad rod, spore positive = Bacillus spp
- Spores are non-swelling and oval shaped ( B. anthracis, B. cereus, B. thuringiensis, B. cereus var mycoides)
- Non-motile: B. anthracis and B.cereus var. mycoides
- Non-hemolytic = Presumptive B. anthracis

**Action:**

If a presumptive B. anthracis is identified, forward isolates to CAREC

Other tests include capsule detection, DFA, gamma phage.

Serological and rapid field tests are not presently available.

**References:**

Basic Laboratory Protocols for the presumptive identification of Bacillus anthracis, Centers for Disease control and Prevention, Atlanta USA.