Warnings

- All specimens received in a biosafety level (BSL) 2 or higher facility are to be processed in a biological safety cabinet (Class II Type A BSC, at a minimum) to adhere to safe BSL2 practices. If a BSC is unavailable in the laboratory, employ an effective splash shield and continue to follow universal precautions. Additional precautions may be necessary if warranted by site-specific risk assessments.

- “Sniffing” of plates is dangerous and should NOT be done. A strong distinctive odor will be apparent without sniffing.

- Wet prep for motility and slide catalase are discouraged, as potential exposure to dangerous pathogens is great. Tube motility and tube catalase are strongly recommended.

- Select Agents are infectious substances that have been determined to have the potential to pose a severe threat to humans. The Category A classification contains both select agents and non-select agents. Shiga-toxin positive E.coli is an example of a Category A non-select agent organism. Additional precautions such as respiratory protection should be added when suspecting a select agent.

Prevent Laboratory Acquired Infections (LAI)

Safety requirements when working with culture plates at an open bench:

- Wear appropriate PPE
- Wash hands frequently
- Keep hands away from nose, mouth and eyes
- Cover cuts and hangnails with adhesive bandages
- Do NOT use personal items in lab (cell phones, lip balm, etc.)
- Immediately seal any plate with mold or fungus
- Never sniff plates!
- Use aerosol-tight rotors for all centrifugations – open rotor with in BSC

- Watch for trigger points!
  - Work up all Gram negative diplococci and coccobacilli seen in original Gram stain of sterile sites in biosafety cabinet (despite likelihood of *Haemophilus influenzae*)
  - Work up all slow growing organisms in biosafety cabinet, especially if no growth or poor growth on MAC. DO NOT USE these organisms on AUTOMATED systems because of lack of accuracy and danger of aerosols
  - Cultures growing suspicious organisms should be manipulated only in the biosafety cabinet – use class II biosafety cabinet with BSL-3 precautions including respiratory protection
A trigger point is a recognized combination of diagnostic findings used to determine when to heighten precautions for handling a specimen or culture.

Trigger points are indicators of possible high-risk pathogens that may require manipulation in a biosafety cabinet (BSC):

- Patient history of travel, hunting, farming, immigration
- Growth from sterile sites - Blood, CSF, Body Fluid
- Gram stain of clinical specimen:
  - Sterile site with Gram negative diplococci or coccobacilli
  - Large Gram positive rods
  - Many WBC, no organisms seen
- Poor growth after 48-72 hours incubation
- Growth only on chocolate or better growth on chocolate compared to SBA
- Growth of Gram negative rod (GNR) or coccobacilli (GNCB) on SBA/Choc with no or poor growth on MacConkey
- Any culture with mold
- Rapid growth of flat, nonhemolytic, irregular colonies with comma projections and ground-glass appearance; Gram stain shows large Gram positive rods, may decolorize.
- GNR with Bipolar staining (safety pin shape) in Gram stain
- GNR with “Fried Egg” or “Hammered Copper” appearance in older cultures
## HAZARDOUS PATHOGENS WORKUP TABLE
### Select Agents

<table>
<thead>
<tr>
<th>Gram Stain Morphology</th>
<th>Growth at 35°C</th>
<th>Rule out</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sheep Blood Agar</td>
<td>Chocolate</td>
</tr>
<tr>
<td><strong>Gram Positive Rods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Large Gram positive rods (1-1.5 μm x 3-5μm), may be in chains</td>
<td>• Good growth at 15-24 hr, growth may be observed as early as 8 hr</td>
<td>See SBA</td>
</tr>
<tr>
<td>• Capsule may be seen as a clear area around the rod in direct clinical specimen</td>
<td>• 2-8 mm flat or slightly convex colonies with irregular edges</td>
<td></td>
</tr>
<tr>
<td>• Gram stain of culture may show subterminal or centralized spores with no significant swelling of the cell</td>
<td>• Ground glass appearance</td>
<td></td>
</tr>
<tr>
<td>• May be easily decolorized</td>
<td>• May have comma shape projections (Medusa head)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• No or very little hemolysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Tenacious – stands up like beaten egg white</td>
<td></td>
</tr>
<tr>
<td><strong>Gram Negative Rods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Gram negative slender rod (0.8 x 2-5 μm) small, straight or slightly curved</td>
<td>• Poor growth at 24 hr</td>
<td>See SBA</td>
</tr>
<tr>
<td>• May demonstrate bipolar (safety pin) morphology.</td>
<td>• Smooth, white, nonpigmented colonies at 48 hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• May become dry, wrinkled colonies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Growth at 42°C</td>
<td></td>
</tr>
<tr>
<td>• Gram negative plump rod (0.5-0.8 x 1-3 μm)</td>
<td>• Pinpoint growth at 24 hr</td>
<td>See SBA</td>
</tr>
<tr>
<td>• Single, short chains in broth</td>
<td>• Gray-white to opaque, nonhemolytic colonies at 48 hr</td>
<td></td>
</tr>
<tr>
<td>• Bipolar stain (safety pin) may occasionally be seen</td>
<td>• May have a “fried egg” appearance after 48-72 hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Growth at room temperature (25°C)</td>
<td></td>
</tr>
</tbody>
</table>

**Summary 1**

WORK UP ALL SLOW GROWING, GRAM NEGATIVE ORGANISMS IN A CLASS II BSC
<table>
<thead>
<tr>
<th>Gram Stain Morphology</th>
<th>Growth</th>
<th>Rule out</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram Negative Coccobacilli or Small Gram Negative Rods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Tiny, pleomorphic, poorly stained Gram negative coccobacillus (0.2x0.2-0.7 µm)</td>
<td>• May initially grow on SBA if cultured from nutrient rich specimen (blood culture)</td>
<td>• Slow growth at ( \leq 48 \text{ hr} )</td>
</tr>
<tr>
<td>• Mostly single cells</td>
<td>• Usually no growth upon subculture - requires cysteine supplementation</td>
<td>• Gray-white, opaque, shiny or wet colonies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sheep Blood Agar</th>
<th>Chocolate</th>
<th>MAC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Slow/no growth at 24 hr</td>
<td>See SBA</td>
<td>No growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Smooth, convex, nonpigmented, nonhemolytic at 48 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

• Small Gram negative coccobacillus (0.5x0.6-1.5 µm), faintly staining
• May be slow to decolorize, can appear as Gram positive cocci

| • Slow/no growth at 24 hr | See SBA | No growth | **Brucella** |
| • Smooth, convex, nonpigmented, nonhemolytic at 48 hr | | | |

• Straight or slightly curved small Gram negative rods or coccobacilli (0.5 x 1.5-3 µm)
• May have rounded ends or wavy sides
• May be in parallel bundles

| • Slow/no growth at 24 hr | See SBA | Poor growth or no growth on MAC | **Burkholderia mallei** |
| • Smooth/gray, translucent colonies at 48 hr | | | |
| • No growth at 42°C in 48 hr | | | |

WORK UP ALL SLOW GROWING, GRAM NEGATIVE ORGANISMS IN A CLASS II BSC

**Summary 2**
<table>
<thead>
<tr>
<th>Gram Stain Morphology</th>
<th>Growth</th>
<th>Rule out</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Select Agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gram Negative Diplococci</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Gram negative cocci in singles or pairs (1-2 μm)</td>
<td>• Smooth, entire edges about 1mm diameter at 18 hr</td>
<td>(See SBA)</td>
</tr>
<tr>
<td>• Possibly intracellular in PMN’s</td>
<td>• Gray, convex, glistening, occasionally mucoid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Blood agar beneath colony may display gray/green color</td>
<td></td>
</tr>
<tr>
<td><strong>Mycobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Faint staining “ghost-like” beaded Gram positive bacilli</td>
<td>• Rapid growing Mycobacteria sp. appear as tiny, dry or “chalky” colonies within 3-5 days; branching filaments may be present on periphery of colonies</td>
<td>(See SBA)</td>
</tr>
<tr>
<td>• Difficult to stain due to high lipid content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Acid fast staining required (carbol fuchsin and fluorochrome stains)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Slender, slightly curved or straight, rod-shaped organisms (0.2-0.6 x 1-10 μm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
GRAM POSITIVE BACILLI

- Encountered in blood, skin lesion, sputum, CSF, rarely stool
- Large; single or in chains; seen in direct smear; may be easily decolorized
- Usually spores not seen in patient specimen; may be seen after extended growth in vitro
- Capsule may be seen from a direct patient specimen but not seen from culture

**Bacillus anthracis**, Gram stain. Direct smear from blood, 1000x (NPHL)

**Bacillus anthracis**, Gram stain. Direct smear from lung, 1000x (CDC)

**Bacillus anthracis**, Gram stain. Direct smear from blood, 1000x (NPHL)

REFER TO *Bacillus anthracis* Tab 1
**Bacillus anthracis Tab 1**

**Trigger Points**
- **Direct Specimen Gram Stain.**
  - **GRAM POSITIVE BACILLI**
  - Large Gram positive rods may appear to be encapsulated (1 - 1.5μm x 3 - 5μm)

**Growth on Sheep Blood Agar**
- Large colonies (2-8mm) < 24 hr
- Flat to slightly convex, irregular edge, comma projections (medusa head), ground glass surface, tenacious

**Hemolysis**
- SBA - 24 hr, No Hemolysis (CDC)

**No Hemolysis**
- Perform all colony manipulations in a BSC

**See Bacillus anthracis flowchart on next tab**
**Bacillus anthracis Tab 2**

Wet prep Motility, India Ink and Slide Catalase

**NOT RECOMMENDED**

Perform all colony manipulations in a BioSafety Cabinet

- **Tube CATALASE**
  - **NEGATIVE**
  - **POSITIVE**

- **Tube MOTILITY**
  - **POSITIVE**
  - **NEGATIVE or questionable**

**Ruled Out Bacillus anthracis**
Continue laboratory identification procedures

- Easily decolorized; may show central to subterminal spores, No significant swelling of cell (CDC)
- Motility Left - Positive, Right - Negative (NPHL)

**Gram Stain from colony - single or chaining GPR, may see central to subterminal spores with no significant swelling of cells. Easily decolorized. Colony consistency - tenacious (beaten egg white)**

**CAN NOT RULE OUT Bacillus anthracis**
CONTACT NPHL at 402-888-5588

*Perform all colony manipulations in a BioSafety Cabinet*
**GRAM NEGATIVE ROD**

**Burkholderia pseudomallei**
- Cause of melioidosis, presents as pneumonia and septicemia systemic with wide-spread abscesses in lungs, liver, spleen and kidney
- Encountered in bone marrow or blood, tissue, urine or respiratory specimens
- Small, straight or slightly curved Gram negative rods
- May demonstrate bipolar staining, resembling safety pins, however this is not relied upon for a presumptive clinical diagnosis

**Yersina pestis**
- Cause of plague, presents as bubonic, septicemic or pneumonic; sudden onset of fever, weakness, painful swollen lymphnodes; extremities turn black. Transmitted by flea bite
- Encountered in blood, lymph node aspirate, respiratory tract
- Medium-sized; plump; mostly single cells, short chains in broth
- Stains well; bipolar “safety pin” stain may occasionally be seen with Wright or Giemsa stain, however, this is not reliable; hard to see on Gram stain

![B. pseudomallei](ASM)  
**B. pseudomallei**  
Gram stain, 1000x  
(ASM)

![Yersinia pestis](CDC)  
**Yersinia pestis**  
Gram stain, 1000x  
(CDC)

**Note:** Bipolar staining reported with other enteric bacteria, e.g., *Pasteurella spp*, Enteric GNR, other *Yersinia spp.*

REFER TO *Burkholderia pseudomallei* Tab  
REFER TO *Yersinia pestis* Tab

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Gram Negative Rod
**Burkholderia pseudomallei**

### Trigger Points

- **Direct Specimen Gram Stain.**
  - **GRAM NEGATIVE RODS**
  - GNR, slender, straight or slightly curved in direct specimen gram stain. May appear bipolar.

- **Sheep Blood Agar - 24 hr (CDC)**
- **Sheep Blood Agar - 72 hr (CDC)**
- **MacConkey Plate - 48 hr (CDC)**

- **Growth on MAC?**
  - **YES**
    - Perform all colony manipulations in a BioSafety Cabinet
    - Gram Stain on colony - GNR may demonstrate bipolar staining, long parallel bundles or irregular shapes.
  - **NO**

- **WARNING!**
  - Commercial Systems should NOT be attempted. May key out as *B. cepacia, B. thailandensis, Ps. aeruginosa, Ps. fluorescens, S. maltophilia, Ps. alcaligenes, C. violaceum*

### Optional Testing

- Oxidase: positive
- Tube Catalase: positive
- Indole: negative
- Tube Motility: positive

### Ruled Out

- **Burkholderia pseudomallei**
- Continue laboratory identification procedures

### CAN NOT RULE OUT **Burkholderia pseudomallei**

- **CONTACT NPHL at 402-888-5588**

---

**Burkholderia pseudomallei**
**Yersinia pestis**

**Trigger Points**

- Direct Specimen Gram Stain. **GRAM NEGATIVE RODS**
  - Medium-size, plump, short chains in broth/blood culture media, may appear bipolar.

- Pinpoint - gray-white, translucent colonies on SBA/Chocolate at 24 hr. Small non-lactose fermenter on MAC at 48 hr. Grows at 25°C. Older cultures may be opaque with “Fried Egg” or “Hammered Copper” appearance.

- Perform all colony manipulations in a Biosafety Cabinet

- Gram stain on colony - plump Gram negative rod, may see bipolar staining.

- Oxidase: negative
  - Tube Catalase: positive
  - Indole: negative
  - Urease: negative
  - 35°/25°C Tube Motility: negative

- YES

**Ruled Out Yersinia pestis**

- Continue identification per routine laboratory procedures

**NO**

- Consider other GNR

**WARNING!**

- Commercial Systems should NOT be attempted. May Key Out As H₂S-negative *Salmonella*, *Shigella*, *Acinetobacter*, *Yersinia pseudotuberculosis*, *Pantoea agglomerans*

**YES**

- Y. pestis, “Fried Egg” Appearance
  - Sheep Blood Agar - 72 hr (CDC)

**CAN NOT RULE OUT Yersinia pestis**

- CONTACT NPHL at 402-888-5588
**GRAM NEGATIVE COCCOBACILLI**

**Francisella tularensis**
- Cause of tularemia, multiple presentations, sudden onset, persists for weeks if not treated
- Encountered in blood, CSF, lymph node, respiratory, abscess/wound, tissue
- Very tiny GNCB, weakly staining, difficult to see individual cells
- Interpretation very difficult due to minute size, often reported as NOS

**Brucella spp.**
- Cause of brucellosis, presentation is non-specific and systemic, with fever, sweats, fatigue, muscle weakness, weight loss, can become chronic
- Encountered in blood, lymph node, bone marrow, liver or spleen, joint fluid, abscess
- Small GNCB, faint but discrete cells will be evident in direct smear
- May retain crystal violet stain, can be mistaken for Gram positive cocci
- 10 days sufficient incubation time in automated blood culture system; 21 days for manual

**Burkholderia mallei**
- Cause of glanders, presents as cutaneous with lymphadenitis or systemic, manifesting as pneumonia or lesions in spleen and liver, often fatal if not treated
- Encountered in bone marrow or blood, respiratory, tissue, abscess/wound specimens or urine
- Faintly staining Gram negative, straight or slightly curved rod with rounded ends or coccobacilli
- May be arranged in pairs end-to-end, in parallel bundles

**REFER TO** Francisella tularensis, Brucella spp or Burkholderia mallei Tab

---

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Gram Negative Coccobacilli
Francisella tularensis

Note: Tularemia is a common laboratory acquired infection; all work on suspect cultures should be performed under BSL2 conditions

Trigger Points

Direct Specimen Gram Stain.
**GRAM NEGATIVE COCCOBACILLI**
NOS or tiny Gram negative coccobacilli. Interpretation may be difficult (smaller than *Haemophilus influenzae*).

**YES**

Poorest growth on Choc at 24 hr. Blue-white to gray, flat, smooth, shiny colonies at 48 hr or more. May initially show poor growth on SBA. Subsequent passages to SBA may fail to grow.

**Perform all colony manipulations in a BioSafety Cabinet**

Gram Stain on colony - faintly staining GNBC, very tiny.

**Oxidase: negative**
**Tube Catalase: weak positive**
**B-Lactamase: positive**

**YES**

Optional Testing
**Urease: negative 24h**
**Fails to satellite around**
*S.aureus* on SBA

**YES**

**CONTACT NPHL at 402-888-5588**

**NO**

**Ruled Out**
*F. tularensis*
Consider *Haemophilus*

**WARNING!**
Commercial systems should NOT be attempted. May key out as *Haemophilus influenzae*, *Aggregatibacter*, *Acinetobacter*, *Bordetella*, *Pasteurella*, *Brucella* or low discrimination.

**NO**

**F. tularensis**

**Chocolate – 72 hr**
(CDC)
**Brucella spp.**

Note: Brucellosis is a common laboratory acquired infection; all work on suspect cultures should be performed under BSL2 conditions.

**Trigger Points**

- **Direct Specimen Gram Stain.**
  - **GRAM NEGATIVE COCCOBACILLI**
  - Small, faint, GNCB, discrete cells evident.
  - May retain crystal violet stain.

- **Oxidase:** positive
- **Tube Catalase:** positive
- **Urease:** positive (rapid to 24 hr)

- **CAN NOT RULE OUT Brucella spp.**
  - **WARNING!**
  - Commercial systems should NOT be attempted. May key out as *Ochrobactrum anthropi*, *Psychrobacter*, *Oligella ureolytica*, *Bordetella bronchiseptica*

- **Growth on SBA, Choc may take 48-72 hr. Non-pigmented, non-hemolytic, moist, convex.**

- **Sheep Blood Agar – 72 hr**

- **Sheep Blood Agar – 48 hr.**

- **Gram Stain on colony GNCB**

- **Perform all colony manipulations in a BioSafety Cabinet**

- **Positive Urease – 1 hr (Top)**

- **CONTACT NPHL at 402-888-5588**

**Ruled Out Brucella spp.**

Consider *Francisella*
Burkholderia mallei

**Trigger Points**

Direct Specimen Gram Stain.

**GRAM NEGATIVE COCCOBACILLI OR SMALL ROD**
Faintly staining, slightly curved, arranged in singles, end-to-end pairs, parallel bundles.

- Oxidase: negative or variable
- Tube Catalase: positive
- Indole: negative
- Urease: negative 24 hr
- Tube Motility: negative

**WARNING!**

Commercial systems should NOT be attempted. May key out as *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*.

- Smooth, gray, translucent colonies at 48 hr.
- Poor or no growth MAC?

**YES**

Perform all colony manipulations in a Biosafety Cabinet.

- Gram Stain on colony GNCB or small GNR

**NO**

Optional Testing

- Arginine: positive
- 42°C Growth: negative

**B. mallei Ruled Out**

Consider Brucella

Continue laboratory identification procedures

**CAN NOT RULE OUT**

*Burkholderia mallei*

CONTACT NPHL at 402-888-5588

**Sheep Blood Agar – 24-48 hr (CDC)**
GRAM NEGATIVE DIPLOCOCCI

Neisseria meningitidis

- Cause of Invasive meningococcal disease (IMD) presents as meningitis or acute sepsis, with petechial lesions which coalesce. Mortality 30% with meningococcal septic shock. Complications include arthritis, pericarditis, pneumonia.
- Encountered in CSF, blood, joint aspirates, biopsy. Organism can be carried in pharyngeal area
- Gram negative diplococci seen in direct Gram stain can be intracellular in PMN’s. May resist decolorization.
- Positive direct smears for gram negative diplococci is sufficient for presumptive diagnosis of meningococcal meningitis.

Note: meningococcemia can be a common laboratory acquired infection (LAI); all work on suspect culture should be performed under BSL2 conditions with BSL3 practices.

Trigger Points

YES
- Growth on both SBA / Choc

NO
- N. meningitidis Ruled out
- Continue laboratory identification
- Oxidase
- Negative
  - Follow laboratory protocol or send to reference laboratory for confirmation. *Submit all confirmed N. meningitidis from sterile body site to NPHL

Positive
- Perform all colony manipulations in a Biosafety Cabinet
# SPOT TESTS OF HAZARDOUS ORGANISMS (adapted from CDC)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gram Stain Morphology</th>
<th>Growth</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Indole</th>
<th>Urease</th>
<th>Beta - Lactamase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SBA</td>
<td>Choc</td>
<td>MAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus anthracis</em></td>
<td>GPR</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td><em>Yersinia pestis</em></td>
<td>GNR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>Burkholderia pseudomallei</em></td>
<td>GNR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>Burkholderia mallei</em></td>
<td>GNCR</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>Francisella tularensis</em></td>
<td>GNCR</td>
<td>−V</td>
<td>+</td>
<td>−</td>
<td>NA</td>
<td>−</td>
<td>Weak</td>
<td>+</td>
</tr>
<tr>
<td><em>Brucella spp.</em></td>
<td>GNCR</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>GNDC</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

+ positive; − negative; +V most species/strains positive; −V most species/strains negative; V variable SBA Sheep Blood Agar; Choc = Chocolate Agar; MAC = MacConkey

**Key Tests**
<table>
<thead>
<tr>
<th>Organism</th>
<th>Biosafety Level</th>
<th>Specimen Exposure/Risk</th>
<th>Recommended Precautions for Sentinel Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus anthracis</strong></td>
<td>2</td>
<td>Blood, skin lesion exudates, CSF, pleural fluid, sputum; rarely urine &amp; feces.</td>
<td>BSL2: Activities involving clinical material collection &amp; diagnostic quantities of infectious cultures. BSL3: Activities with high potential for aerosol or droplet production.</td>
</tr>
<tr>
<td><strong>Brucella spp.</strong></td>
<td>2</td>
<td>Blood, bone marrow, CSF, tissue, semen, occasionally urine.</td>
<td>BSL2: Activities limited to collection, transport &amp; plating of clinical material.</td>
</tr>
<tr>
<td><strong>Burkholderia mallei &amp; pseudomallei</strong></td>
<td>2</td>
<td>Blood, sputum, CSF, tissue, abscesses, and urine</td>
<td>BSL2: Activities limited to collection, transport &amp; plating of clinical material.</td>
</tr>
<tr>
<td><strong>Francisella tularensis</strong></td>
<td>2</td>
<td>Skin lesion exudates, respiratory secretions, CSF, blood, urine, tissues from infected animals &amp; fluids from infected arthropods.</td>
<td>BSL2: Activities limited to collection, transport &amp; plating of clinical material.</td>
</tr>
<tr>
<td><strong>Yersinia pestis</strong></td>
<td>2</td>
<td>Bubo fluid, blood, sputum, CSF, feces, urine.</td>
<td>BSL2: Activities involving clinical material collection &amp; diagnostic quantities of infectious cultures. BSL3: Activities with high potential for aerosol or droplet production.</td>
</tr>
</tbody>
</table>
RESOURCES

Packaging and shipping requirements must be met for all samples and are the responsibility of the shipper. Shipping materials can be obtained by calling NPHL at (402) 559-3590. Shipping instructions can be found www.nphl.org

BT preparedness information can be found on the CDC website www.bt.cdc.gov

Biosafety in Microbiological and Biomedical Laboratories (BMBL); DHHS/CDC – 5th Edition, Revised 2009


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