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 µ 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*, procede as outlined below under "Reporting". Otherwise, report as

"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 / cocobaccilli 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".

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