**BIOTHREAT AGENTS**

**ANTHRAX**  
*Bacillus anthracis*  
- Large Gram positive rods (1-1.5 µm x 3-5 µm)  
- Smears of clinical specimens:  
  - Short chains (2-4 cells)  
  - Capsule present, no spores  
- Smears from BAP and CHOC culture:  
  - Long chains, no capsule  
  - Spores in older cultures; oval, central to subterminal, no swelling of cell wall  
- Grows well on BAP and CHOC  
- No growth on MAC and EMB  
- Ground-glass colonies, 2-5 mm on BAP and CHOC at 24h  
- Aerobic growth as early as 4-8h  
- Flat or slightly convex with irregular edges that may have comma-like projections  
- Non-hemolytic on BAP  
- Tenacious, sticky colonies, adheres to agar surface  
- Catalase positive  
- Non-motile

**BRUCELLOSIS**  
*Brucella* spp.  
- Tiny, faintly staining, non-clustered, Gram negative coccobacilli (0.4 µm-0.8 µm)  
- Pinpoint colonies at 24h, and 0.5-1.0 mm after 48h  
- Non-hemolytic  
- Non-mucoid  
- Aerobic growth on BAP and CHOC (CO₂ may be required by some strains)  
- No growth on MAC or EMB  
- Catalase, oxidase, urea: positive (Oxidase may be variable)  
- X and V factor (satellite test) negative (not required)  
- Non-motile (although motility testing not recommended for suspect *Brucella* spp.)  
- Aerobic  
- Non-hemolytic  
- Growth on MAC (may uptake pink dye)  
- Distinctive musty earthy odor, which is diagnostic (the odor is apparent without sniffing)  
- Oxidase positive  
- Spot indole negative  
- Non-motile  
- No growth at 42°C  
- Polymyxin B and colistin no zone  
- Penicillin resistant  
- Amoxicillin-clavulanate susceptible

**GLANDERS**  
*Burkholderia mallei*  
- Small straight, or slightly curved with rounded ends, Gram negative coccobacilli (1.5 µm-3 µm x 0.5-1.0 µm)  
- Cells arranged in pairs, parallel bundles, or Chinese letter form  
- Aerobic  
- Non-hemolytic  
- No growth or pinpoint on MAC at 48h  
- Catalase positive  
- Oxidase variable  
- Spot indole negative  
- Non-motile  
- No growth at 42°C  
- Polymyxin B and colistin no zone  
- Penicillin resistant  
- Amoxicillin-clavulanate susceptible

**MELIOIDOSIS**  
*Burkholderia pseudomallei*  
- Straight, or slightly curved Gram negative rod (0.2-0.5 µm x 0.4-0.8 µm)  
- Colonies may demonstrate bipolar morphology in direct specimens and peripheral staining in older cultures, which can mimic endospores  
- Aerobic  
- Non-hemolytic  
- Growth on MAC (may uptake pink dye)  
- Distinctive musty earthy odor, which is diagnostic (the odor is apparent without sniffing)  
- Oxidase positive  
- Spot indole negative  
- Motile  
- Growth at 42°C  
- Polymyxin B and colistin no zone  
- Penicillin resistant  
- Amoxicillin-clavulanate susceptible

**TULAREMIA**  
*Francisella tularensis*  
- Tiny, Gram negative coccobacilli (0.2-0.5 µm x 0.7-1.0 µm)  
- Poor counterstaining with safranin (basic fuchsin counter-stain may increase resolution)  
- Pleomorphic  
- Mostly single cells  
- Aerobic, fastidious  
- No growth on MAC/EMB  
- Scant or no growth on BAP; may grow on primary culture, not well on subculture  
- Slow growing on CHOC, TM or BCYE: 1-2 mm after 48h  
- Colonies are opaque, grey-white, butyrous, smooth and shiny  
- Oxidase negative  
- Catalase negative or weakly positive  
- Satellite negative  
- Beta-lactamase positive

**PLAGUE**  
*Yersinia pestis*  
- Plump, Gram negative rods (0.5 x 1-2 µm) seen mostly as single cells or pairs, and may demonstrate short chains in liquid media  
- May exhibit bipolar, “safety-pin” appearance in Giemsa stain or Wright’s stain  
- Facultative anaerobe  
- Slow growing at 35°C, better growth at 25-28°C  
- Grey-white, translucent pinpoint colonies at 24h, usually too small to be seen, little to no hemolysis on BAP  
- At 48h, lactose non-fermenter on MAC or EMB  
- Catalase positive  
- Oxidase, urease (at 35°C) and indole negative

---

**FOLLOW ALL LABORATORY AND BIOSAFETY PROCEDURES TO RECOGNIZE AGENTS OF BIOTERRORISM**  
**YOU ARE THE FIRST LINE OF DEFENSE — REFER TO CURRENT ASM SENTINEL LAB PROTOCOLS**