### Anthrax
*Bacillus anthracis*
- **Large Gram positive rods** (1.5-3.5 µm x 1-1.5 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.)

### Glanders
*Burkholderia mallei*
- **Small straight, or slightly curved with rounded ends, Gram negative rod** (2.0-5.0 µm x 0.4-0.8 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
- Motile
- 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** (2.0-5.0 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
- Beta-lactamase positive

### Tularemia
*Francisella tularensis*
- **Tiny, Gram negative cocccobacilli** (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

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**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*