

# *Burkholderia pseudomallei*

*Burkholderia pseudomallei* is an oxidase-positive, aerobic gram-negative bacillus that is straight or slightly curved. The organism will grow on most standard laboratory media, such as sheep blood and chocolate and MacConkey agars, and it produces a characteristic musty odor. Melioidosis, also called Whitmore's disease, is an infectious disease caused by the bacterium *Burkholderia pseudomallei*. Melioidosis is clinically and pathologically similar to glanders disease, but the ecology and epidemiology of melioidosis are different from glanders. Melioidosis is predominately a disease of tropical climates, especially in Southeast Asia where it is endemic. The bacteria causing melioidosis are found in contaminated water and soil and are spread to humans and animals through direct contact with the contaminated source. Glanders is contracted by humans from infected domestic animals. Melioidosis is diagnosed by isolating *Burkholderia pseudomallei* from the blood, urine, sputum, or skin lesions. Detecting and measuring antibodies to the bacteria in the blood is another means of diagnosis.

## Specimens

- Blood or bone marrow
- Sputum or bronchoscopically obtained specimens
- Abscess material and wound swabs
- Urine
- Serum (1 ml). Both acute- and convalescent-phase (obtained 14 days after the acute-phase specimen) specimens should be collected if serologic diagnosis of *B. pseudomallei* infection is being considered.

## Materials

- Blood and bone marrow cultures can be done using:
  - Standard automated blood culture system
  - Lysis centrifugation system
- Media for isolation from other clinical specimens:
  - Chocolate agar (CHOC)
  - Sheep blood agar (SBA)
  - MacConkey agar (MAC)

## Cultures

- Blood cultures - Process according to standard laboratory procedure.
- Respiratory specimens, abscess material/wounds and urine - Plate directly onto SBA and MacConkey agar; enrichment broth can be used for wound/abscess material. For improved isolation, a colistin disk or polymyxin B disk may be placed in the initial inoculation area of the SBA if isolation of *Burkholderia* spp. is specifically requested.

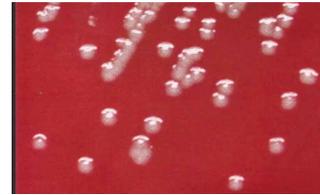
## Culture Incubation

- Temperature - 35 to 37°C
- Atmosphere - Ambient; CO<sub>2</sub> acceptable

- Length of incubation - Hold primary plates for a minimum of 5 days; read daily. *B. pseudomallei* will reliably grow with 5 days of incubation from blood cultures, so extended incubation of either broth or plated blood cultures (lysis centrifugation) is not necessary.

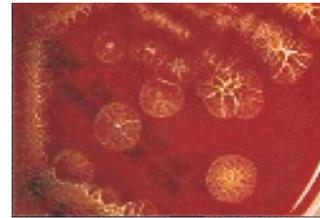
### Colony Morphology

- Smooth, creamy, white colonies on SBA at 24 hrs
- Some may be mucoid or become dry and wrinkled at 48 - 72 hrs
- Pink colonies on MAC at 24 - 48 hrs or colorless colonies at 48 hrs



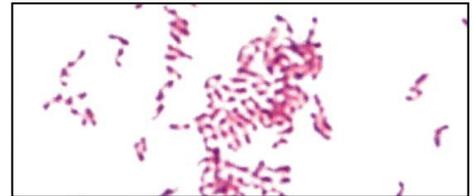
### Gram Stain

- Gram-negative slender rods with bipolar staining (1-3  $\mu\text{m}$  x < 2.0  $\mu\text{m}$ )
- Smooth form appears as long parallel bundles and rough form appears more irregularly arranged

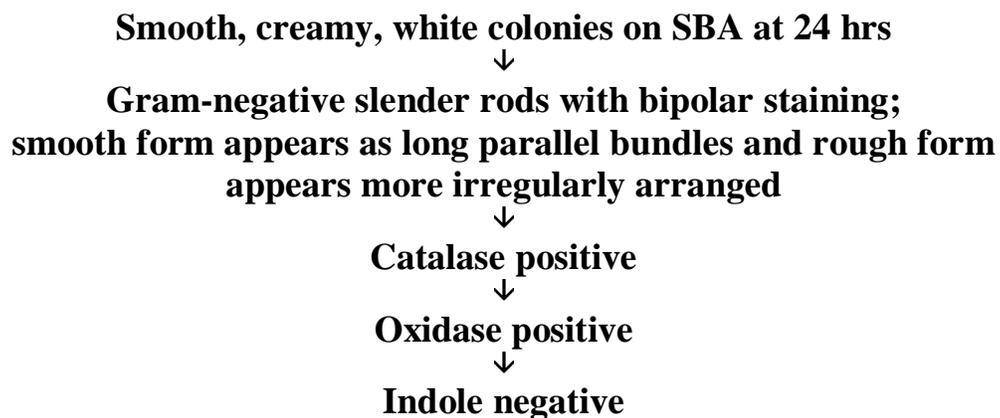


### Biochemical test and reactions

- Catalase = Positive
- Oxidase = Positive
- Indole = Negative
- Motility = Positive
- Triple Sugar Iron (TSI) Agar Slant = Slant: variable; Butt: red (no change)
- Colistin/Polymyxin B = Resistant (no zone)



### Identification Flow Chart





**Motile**



**TSI - Slant: variable; Butt: red (no change)**



**Resistant to Colistin/Polymyxin B**



**Refer to State Laboratory**

**610-280-3464**