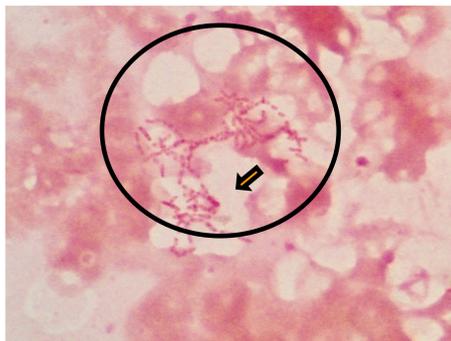


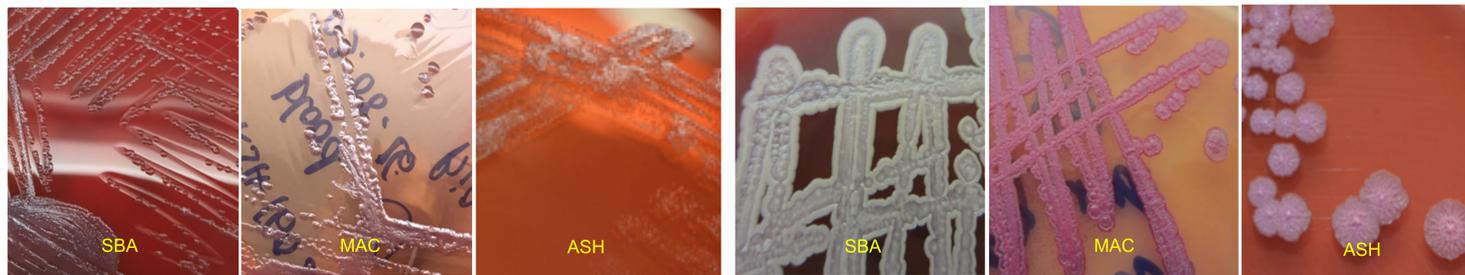
Burkholderia pseudomallei is the causative agent of melioidosis, a serious disease with a high mortality rate. *B. pseudomallei* is endemic in Cambodia, therefore it is critical for bacteriology staff in Cambodian laboratories to recognize and identify this pathogen. This poster describes the methods used for identification, including; Gram stain, colony morphology, biochemical testing and three-disc testing. If available, latex agglutination or InBios Active Melioidosis Detect™ Rapid lateral flow may be useful for reducing turn around time. Note: Perform all culture work in a certified Biosafety Cabinet. Always perform Internal Quality Control to ensure media and biochemical tests and discs are working as expected.

1- Gram stain



Gram negative bacilli with tapered ends. They may appear as bipolar or show irregular staining. Enterobacteriaceae also may have bipolar "safety pin" appearance but are fatter than *B. pseudomallei* and don't taper at the ends.

2- Colony morphology



24 hour incubation in air 35 °C

72 hour incubation in air 35 °C

Sheep Blood agar (SBA): pinpoint to small, metallic, gray
MacConkey (MAC): pinpoint to small, pale pink (non-lactose fermenting)
Ashdown's agar (ASH): pinpoint to small clear or pale pink colonies

SBA: small to medium, metallic, gray-white, wrinkled
MAC: small to medium, pink, wrinkled
ASH: small to medium, pink or purple, metallic, wrinkled.
Variants are possible such as large mucoid colonies and dryer colonies with lighter shades of purple or pink

3- Oxidase test



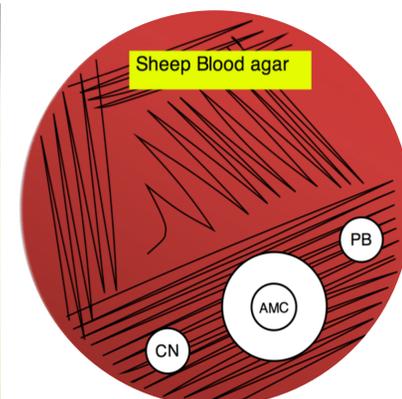
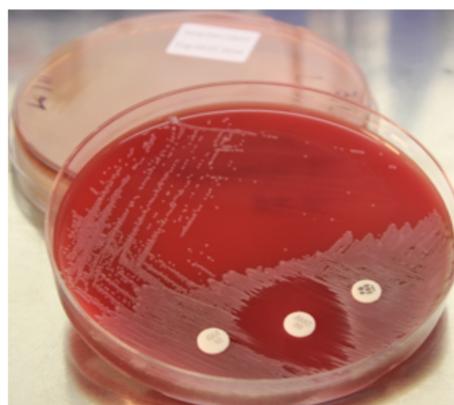
Burkholderia pseudomallei
Oxidase: positive

4- Simple biochemistry test



Kligler Iron Agar (KIA): glucose and lactose non fermenting (alkaline/No change).
Lysine Iron Agar (LIA): No reaction (No change/No change)
Sulphide, Indole, Motility medium (SIM): Sulphide negative, Indole negative (add 2 drops of Kovac's reagent), Motility-surface pellicle, no growth away from stab line (SIM is not the ideal method for detection of motility in *B. pseudomallei*)
Urea: negative (variable)
Simmons Citrate: negative (variable)

5- Simple 3 disc test



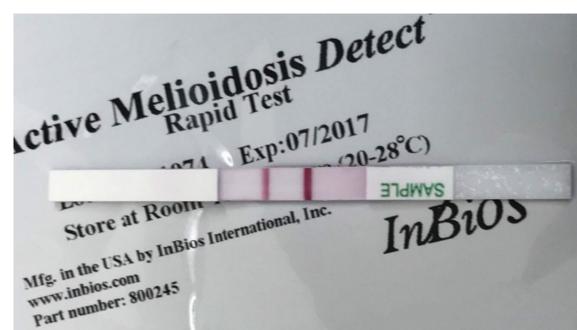
Burkholderia pseudomallei is usually susceptible to Amoxi/Clav, but resistant to Gentamicin and Colistin/Polymixin B.

6- Latex Agglutination test



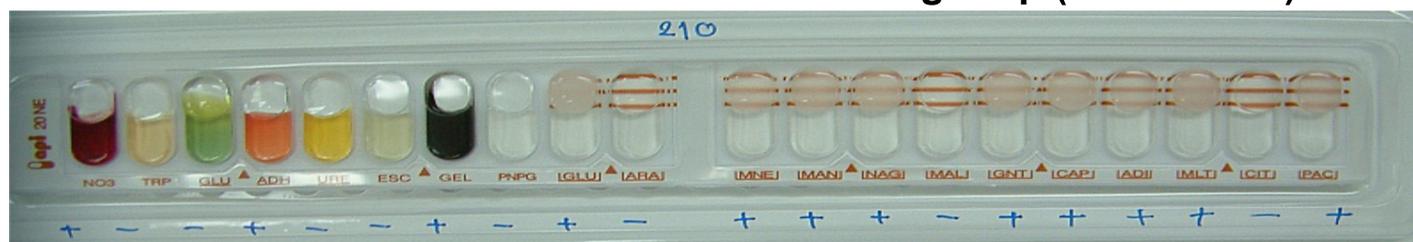
Pick suspect colony and emulsify in reagent, gently rock the solution for 2 minutes before reading the result (positive shows agglutinated particles in a relative clear solution).

7- InBios Active Melioidosis Detect™ Rapid Test



This test can be performed on colonies or direct specimen: e.g. sputum, pus, body fluid, urine and blood culture broth with Gram negative bacilli. The presence of 2 lines (test and control) indicates a positive result regardless of the intensity of the lines (the line may be faint but is still read as positive). The result is read after 15mins

8- Commercial API 20 NE biochemical testing strip (BioMerieux)



Common profile numbers are 1156577, 1556577, 1156576, 1556576, 1156575, 1556575

Note: Be aware that each test is not always perfect. False-positive and false-negative results by a single test are not uncommon. Using multiple methods for confirmation is recommended. Gram-stain showing bipolarity is neither sensitive nor specific.

Any Gram-negative bacillus that is oxidase-positive and not *Pseudomonas aeruginosa* should be suspected potentially to be *B. pseudomallei*