



Test Name	Melioidosis (<i>Burkholderia pseudomallei</i>) PCR
Specimen Type	<ol style="list-style-type: none"> 1. Expecterated sputum, endotracheal aspirates, broncho-alveolar lavage Collect at least 0.5 ml in sterile container. 2. Urine Collect at least 0.5 ml in sterile container. 3. Swab Only dry Dacron swabs or eSwabs will be accepted. Swabs in Amies gel media will not be processed. 4. Tissue Place a piece of FRESH sample of 5x5x5 mm directly in a sterile container. 5. Pus and other fluids Collect at least 0.5 ml in sterile container. 6. Blood (EDTA tube only) Collect at least 2ml blood in 1 EDTA tube
Special Instructions For Laboratory	-
Specimen Storage And Transport	Store specimen at 4°C. Transport to laboratory as soon as possible.
Specimen Minimum Volume	0.5 ml for liquid specimens 2 ml for blood specimens
Test Method	<p>Real-time PCR, lab developed test (non-FDA approved).</p> <p>This assay involves real-time polymerase chain reaction (PCR) targeted at the BPSS1187/BURPS1710b_A0179 hypothetical protein gene and orf2 of TTS1 gene of the bacteria.</p> <p>Reference</p> <p>Supaprom, C., D. Wang, C. Leelayuwat, W. Thaewpia, W. SUSAENGAT, V. Koh, E. E. Ooi, G. Lertmemongkolchai, and Y. Liu. 2007. Development of real-time PCR assays and evaluation of their potential use for rapid detection of <i>Burkholderia pseudomallei</i> in clinical blood specimens. J Clin Microbiol 45:2894-901.</p> <p>Ryan T. Novak, Mindy B. Glass, Jay E. Gee, Daniel Gal, Mark J. Mayo, Bart J. Currie, and Patricia P. Wilkins. 2006. Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of <i>Burkholderia pseudomallei</i>. J Clin Microbiol 44:85-90.</p>
Expected Result	Detected, Not detected, Inhibitory
Reference Ranges	Refer to Lab report
Turn Around Time	1-3 days
Days Of Testing	Monday - Saturday
Hospital	CGH



Laboratory	Microbiology Lab
Discipline	Microbiology
Contact Details	4935 / 4936
CPOE Order Name Synonyms	Melioidosis (B. pseudomallei) PCR
Clinical Information	<p>APPROPRIATE CLINICAL USAGE Patients with suspected infection caused by <i>Burkholderia pseudomallei</i>.</p> <p>A few studies recommend routinely performing blood, throat and urine cultures for all patients with suspected melioidosis, regardless of their symptoms. A separate publication from Northern Australia (using a different PCR assay) has documented that sputum and urine are useful clinical samples for diagnostic PCR testing in patients with melioidosis. The same study demonstrated that it was not unusual for sputum and urine to be PCR positive in specimens from melioidosis patients which were culture negative. Other studies have documented that throat swabs for <i>B. pseudomallei</i> culture may be helpful for the diagnosis of invasive melioidosis, with a specificity of 100% and sensitivity of 20-40%.</p> <p>The technical limit of detection of this assay for blood samples (EDTA) is ~90 cfu/ml.* However, the clinical performance of this assay for detecting true bacteraemia is not defined, given that the estimated burden of organisms in blood may vary from 1 to 100 cfu/ml. Blood cultures remain the gold standard for diagnosis of bacteraemia.</p> <p>LIMITATIONS OF ASSAY Bacterial nucleic acids may persist in vivo independent of organism viability. Detection of organism target(s) does not imply that the corresponding organisms are infectious. The performance of the this test has not been established for monitoring treatment of infection.</p>
Link Out For Additional Information	-
Remarks	<p>Performance Characteristics</p> <ol style="list-style-type: none"> EFFICIENCY AND LINEAR RANGE The dual-target assay covers a linear range from 30 CFU per PCR reaction to at least 3×10^5 CFU per PCR reaction. The PCR efficiency of gene target A0179 is 96%, and the PCR efficiency of gene target TTS-1 is 93%. LIMIT OF DETECTION 20 replicates of DNA sample (containing 5 CFUs per PCR reaction) were subjected to the PCR assay. 18/20 (90%) of replicates were detected by A0179 gene target. 20/20 (100%) of the replicates were detected by TTS-1 gene target. The LOD of this assay is 5 CFU per PCR reaction. Spiked EDTA blood sample (90 CFU/ml of blood) was subjected for DNA extraction and then the PCR assay. 16/20 (80%) of replicates were detected by A0179 gene target. 20/20 (100%) of the replicates were detected by TTS-1 gene target. The LOD of this assay for blood sample is at least 90 CFU/ml of blood, equals to 12 CFU per PCR reaction. SENSITIVITY Cultural positive bacterial colonies (n=25) were gone through heat inactivation, DNA extraction and PCR. The PCR results were compared with culture results.



The analytical sensitivity is 100%.

Cultural positive clinical samples, BAL (n=5), EDTA blood (n=1), ETT aspirate (n=7), joint fluid (n=2), meninges tissue (n=1), neck aspirate (n=1), pleural fluid (n=1), prostatic aspirate (n=2), spleen aspirate (n=4), sputum (n=2) were gone through DNA extraction and PCR. The multiplex PCR results were compared with simplex PCR results and/or culture results. The clinical sensitivity is 100%.

4. SPECIFICITY

The assay was tested for potential cross-reactivity against the following panel of bacterial samples (n=25). No cross-reactivity was observed. The analytical specificity is 100%.

Acinetobacter baumannii complex
Aeromonas hydrophilia/cariae
Burkholderia cenocepacia
Burkholderia Cepacia
Burkholderia thailandensis
Chryseobacterium (elizabethkingia) meningoseptica
Comamonas testosteroni
Corynebacterium simulans
Corynebacterium striatum
Enterobacter aerogenes
Escherichia coli
Haemophilus influenzae
Klebsiella oxytoca
Moraxella catarrhalis
Mycobacterium sp (RGM)
Proteus mirabilis
Pseudomonas aeruginosa
Sphingomonas Paucimobilis
Staphylococcus aureus
Staphylococcus epidermidis
Stenotrophomonas maltophilia
Streptococcus mitis/oralis

The assay was tested for potential cross-reactivity against the following clinical samples (n=26). No cross-reactivity was observed. The clinical specificity is 100%.

Sample Type	Culture Results
BAL	<i>Acinetobacter baumannii complex, Pseudomonas aeruginosa</i>
BAL	<i>Haemophilus influenzae</i>
BAL	<i>Klebsiella pneumoniae ssp pneumoniae</i>
ETTA	<i>Acinetobacter iwoffii</i>
ETTA	<i>Burkholderia Cepacia</i>
ETTA	<i>Candida albicans</i>
ETTA	<i>Citrobacter koseri, Group G Streptococcus, Pseudomonas aeruginosa</i>
ETTA	<i>Group C Streptococcus</i>
ETTA	<i>Haemophilus influenzae</i>
ETTA	<i>Klebsiella pneumoniae ssp pneumoniae</i>
ETTA	<i>Moraxella catarrhalis</i>
ETTA	<i>Pseudomonas aeruginosa, Klebsiella pneumoniae</i>
ETTA	<i>Pseudomonas aeruginosa, Stenotrophomonas maltophili</i>
ETTA	<i>Staphylococcus aureus</i>
Sputum	<i>Acinetobacter baumannii complex</i>
Sputum	<i>Burkholderia Cepacia</i>
Sputum	<i>Enterobacter cloacae complex</i>
Sputum	<i>Methicillin-resistant S. auerus</i>
Sputum	<i>Serratia marcescens</i>
Sputum	<i>Stenotrophomonas maltophilia</i>



Urine	<i>Candida species</i>
Urine	<i>Citrobacter koseri</i>
Urine	<i>Enterococcus species</i>
Urine	<i>Escherichia coli</i>
Urine	<i>Staphylococcus aureus</i>
Urine	<i>Streptococcus Group B</i>