Joint Biological Agent Identification and Diagnostic System (JBAIDS)

Burkholderia Analytical Studies and Data Package

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Background

A rapid and reliable identification of *Burkholderia* species is essential. *Burkholderia* is responsible for devastating diseases, such as Glanders and melioidosis, and is the causative agent in many hospital-acquired infections. The severity of the disease and the difficulties with its diagnosis led the CDC to list it as a Category B Biological Disease. *Burkholderia* spp. are known to exchange plasmid DNA frequently. As a result of this genomic plasticity, creating an assay capable of detecting both *Burkholderia mallei* and *Burkholderia pseudomallei* has been problematic. This study will evaluate the PB1 nucleic acid-based detection assay for the feasibility of developing an IVD test kit for the JBAIDS platform to be used by the Department of Defense (DoD).

Funded by the Chemical Biological Medical Systems (CBMS), this study outlines an analysis that assessed the improved assay for detection and identification of *Burkholderia mallei* (BURK014/BURK010) and *Burkholderia pseudomallei* (BURK003) in clinical human serum samples. The Pan-*Burkholderia* assay (PB1) underwent analytical testing to include Limit of Detection (LoD), inclusivity and exclusivity testing, and mock clinical trials. Data generated from this study is intended to use for the development of a data package consistent with the CB.56 Molecular Assay Transition Package with consideration for the Review of Memorandum for Pre-Emergency Use Authorization (Pre-EUA).

Test Principle

LoD Determination:

Using live antigen and genomic DNA, the analytical sensitivity LoD determined the lowest detectable concentration at which 95% of all replicates tested positive both in pristine buffer and human serum. All test samples were extracted using the QIAamp DNA Mini Kit.

LoD Results:

Organism	Matrix	GE/mL	Results	Mean Cp	SD	%CV
Live *Burkholderia mallei*	Serum	5,000	40/40	35.98	1.87	5.20
Live *Burkholderia pseudomallei*	Serum	5,000	38/40	37.87	2.70	7.14
Inactivated *Burkholderia mallei*	Serum	30,000	24/24	32.41	0.87	2.69
Inactivated *Burkholderia pseudomallei*	Serum	5,000	24/24	34.03	0.51	1.51

Inclusivity/Exclusivity:

Inclusivity (1x LoD) and exclusivity (1,000x LoD) testing was performed using a panel of genomic DNAs from various *Burkholderia* species, and other organisms closely and not closely related.

Purified DNA from Bacteria	CRP Cat. Number	Concentration (pg DNA/Rxn)	C_p Value		mean C_p Value (n=2)
Burkholderia pseudomallei 1026B	DNA-BURK003	0.1845	42.00	35.83	38.92
Burkholderia mallei (SR092700A)	DNA-BURK005	1.482	34.45	34.81	34.63
Burkholderia mallei (SR092700C)	DNA-BURK007	1.482	35.70	42.00	38.85
Burkholderia mallei (SR092700E)	DNA-BURK009	1.482	34.78	34.52	34.65
Burkholderia mallei 23344	DNA-BURK010	1.482	32.47	32.64	32.56
Burkholderia mallei	DNA-BURK012	1.482	32.88	34.24	33.56
Burkholderia mallei	DNA-BURK014	1.482	35.44	36.03	35.74
Burkholderia mallei (Turkey 1)	DNA-BURK062	1.482	33.68	33.58	33.63
Burkholderia mallei (Turkey 2)	DNA-BURK063	1.482	34.09	34.65	34.37
Burkholderia mallei (Turkey 3)	DNA-BURK064	1.482	34.13	33.77	33.95
Burkholderia mallei (Turkey 4)	DNA-BURK065	1.482	34.00	33.15	33.58
Burkholderia mallei (Turkey 5)	DNA-BURK066	1.482	34.18	34.31	34.25
Burkholderia mallei (Turkey 6)	DNA-BURK067	1.482	34.28	33.69	33.99
Burkholderia mallei (Turkey 7)	DNA-BURK068	1.482	34.02	33.24	33.63
Burkholderia mallei (Turkey 8)	DNA-BURK069	1.482	33.46	35.10	34.28
Burkholderia mallei (Turkey 9)	DNA-BURK070	1.482	33.58	34.52	34.05
Burkholderia mallei (85-567)	DNA-BURK073	1.482	33.60	34.75	34.18
Burkholderia mallei China 5	DNA-BURK077	1.482	34.13	35.97	35.05
Burkholderia mallei	DNA-BURK078	1.482	35.17	34.99	35.08
Burkholderia mallei (2002721279)	DNA-BURK082	1.482	35.72	36.33	36.03
Burkholderia mallei (Turkey10)	DNA-BURK084	1.482	36.12	35.12	35.62
Burkholderia pseudomallei	DNA-BURK087	0.1845	35.67	38.04	36.86
Burkholderia pseudomallei	DNA-BURK088	0.1845	36.91	36.33	36.62
Burkholderia pseudomallei	DNA-BURK098	0.1845	35.95	37.77	36.86
Burkholderia pseudomallei K96243	DNA-BURK119	0.1845	38.36	42.00	40.18
Burkholderia mallei strain 11	DNA-BURK131	1.482	35.46	34.00	34.73
Burkholderia pseudomallei 406e	DNA-BURK132	0.1845	34.12	35.64	34.88
Burkholderia pseudomallei 112	DNA-BURK133	0.1845	36.37	37.38	36.88
Burkholderia pseudomallei 9	DNA-BURK134	0.1845	42.00	38.12	40.06
Burkholderia pseudomallei 1106a	DNA-BURK142	0.1845	42.00	42.00	42.00

Purified DNA from Bacteria	CRP Cat. Number	Concentration (pg DNA/Rxn)	C_p Value
Burkholderia cepacia	DNA-BURK001	1482	N/D
Burkholderia gladioli gladioli	DNA-BURK042	1482	N/D
Burkholderia multivorans	DNA-BURK101	1482	N/D
Burkholderia cepacia	DNA-BURK102	1482	N/D
Burkholderia cepacia	DNA-BURK106	1482	N/D
Burkholderia cenocepacia	DNA-BURK108	1482	N/D
Burkholderia thailandensis CDC3015869	DNA-BURK113	1482	N/D
Burkholderia oklahomensis E0147	DNA-BURK114	1482	N/D
Burkholderia oklahomensis C6786	DNA-BURK115	1482	N/D
Burkholderia vietnamiensis	DNA-BURK126	1482	N/D
Burkholderia dolosa	DNA-BURK138	1482	N/D
Burkholderia spp. (MSMB43)	DNA-BURK161	1482	N/D
Burkholderia ubonensis	DNA-BURK162	1482	N/D
Burkholderia spp. (MSMB121)	DNA-BURK164	1482	N/D

Cross Reactivity:

Bacterial cross-reactivity of the PB1 assay was evaluated by testing purified nucleic acid from antigens that potentially infect the majority of the population. Cross-reactivity was conducted at 1,000x LoD.

Purified DNA from Bacteria	CRP Cat. Number	Concentration (pg DNA/Rxn)	C_p Value
Acinetobacter baumanni 19606	DNA-ACIN001	1482	N/D
Bacillus coagulans NRS 609	DNA-BACI020	1482	N/D
Bacillus licheniformis	DNA-BACI021	1482	N/D
Bacillus macerans	DNA-BACI025	1482	N/D
Bacillus megaterium	DNA-BACI026	1482	N/D
Bacillus mycoides 10206	DNA-BACI028	1482	N/D
Bacillus subtilis v. niger (globigii)	DNA-BACI051	1482	N/D
Bacillus thuringiensis Kurstaki	DNA-BACI204	1482	N/D
Comamonas acidovorans 15668	DNA-COMA003	1482	N/D
Corynebacterium sp.	DNA-CORY001	1482	N/D
Enterobacter agglomerans 29904	DNA-ENTB002	1482	N/D
Enterobacter cloacae 13047	DNA-ENTB004	1482	N/D
Enterococcus faecalis 29212	DNA-ENTC002	1482	N/D
Erysipelothrix rhusiopathiae	DNA-ERYS001	1482	N/D
Haemophilus influenzae 10211	DNA-HAEM001	1482	N/D
Klebsiella pneumoniae 13883	DNA-KLEB001	1482	N/D
Listeria monocytogenes	DNA-LIST001	1482	N/D
Moraxella catarrhalis	DNA-MORA001	1482	N/D
Proteus vulgaris 49132	DNA-PROT002	1482	N/D
Providencia stuartii 33672	DNA-PROV001	1482	N/D
Salmonella enteritidis	DNA-SALM001	1482	N/D
Serratian marcescens CDC-813-60	DNA-SERR003	1482	N/D
Shigella sonnei	DNA-SHIG002	1482	N/D
Staphylococcus epidermidis	DNA-STAP009	1482	N/D
Stenotrophomonas maltophilia	DNA-STEN001	1482	N/D
Streptococcus pyrogenes Rosenbach	DNA-STRE010	1482	N/D
Yersinia pseudotuberculosis 6904	DNA-YERS008	1482	N/D
Yersinia enterocolitica	DNA-YERS014	1482	N/D

Mock Clinical:

A mock clinical trial was conducted using inactivated *B. mallei* and *B. pseudomallei* and spiked in a blinded fashion using three concentrations (1.5x, 3.0x, and 1,000x LoD) in human serum.

PB1 Detection Assay on the JBAIDS Platform	Positive Results		Negative Results
Positive Samples (54)	54		0
Negative Samples (100)	0		100
Total (154)	54		100
[redacted]			
			95 % CI
Positive Percent Agreement	54/54	100%	96.2%-98.5%
Negative Percent Agreement	100/100	100%	99.3-100%

Conclusions

The PB1 assay effectively detected *B. mallei* and *B. pseudomallei* at relatively low concentrations. This assay has the potential to be used in Pre-Emergency Use Authorization purposes should the need arise.

Approved for Public Release

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