

Real-time PCR (RT-PCR) Assays for *Burkholderia mallei* and *B. pseudomallei*

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ABSTRACT

Burkholderia pseudomallei and *B. mallei*, respectively are the causative agents of melioidosis and glanders, primarily in animals (both pathogens), and in humans (commonly the former but sporadically the latter as well). The two pathogens are gram-negative, facultative anaerobe, motile bacilli. There is no known vaccine, and the treatment with antimicrobials is protracted because of natural resistance of these pathogens to commonly used antibiotics. The virulence factors are only now beginning to be elucidated and understood (Rockseidler *et al.*, 2001, Infection and Immunity, 69: 34-44). These pathogens, consequently, are viewed as a serious public health concern.

Early detection of these threat pathogens is critical to minimize the potential risk. There is currently no real-time PCR assay for detection of both of these pathogens. Primers and probes corresponding to specific genomic regions were designed and tested in an attempt to develop RT-PCR assays for detection of both these pathogens. Here, we report successful development of highly specific RT-PCR assays for detection of both *B. mallei* and *B. pseudomallei* cells. To the best of our knowledge, this work constitutes the first report of RT-PCR assays for detection of these pathogens.

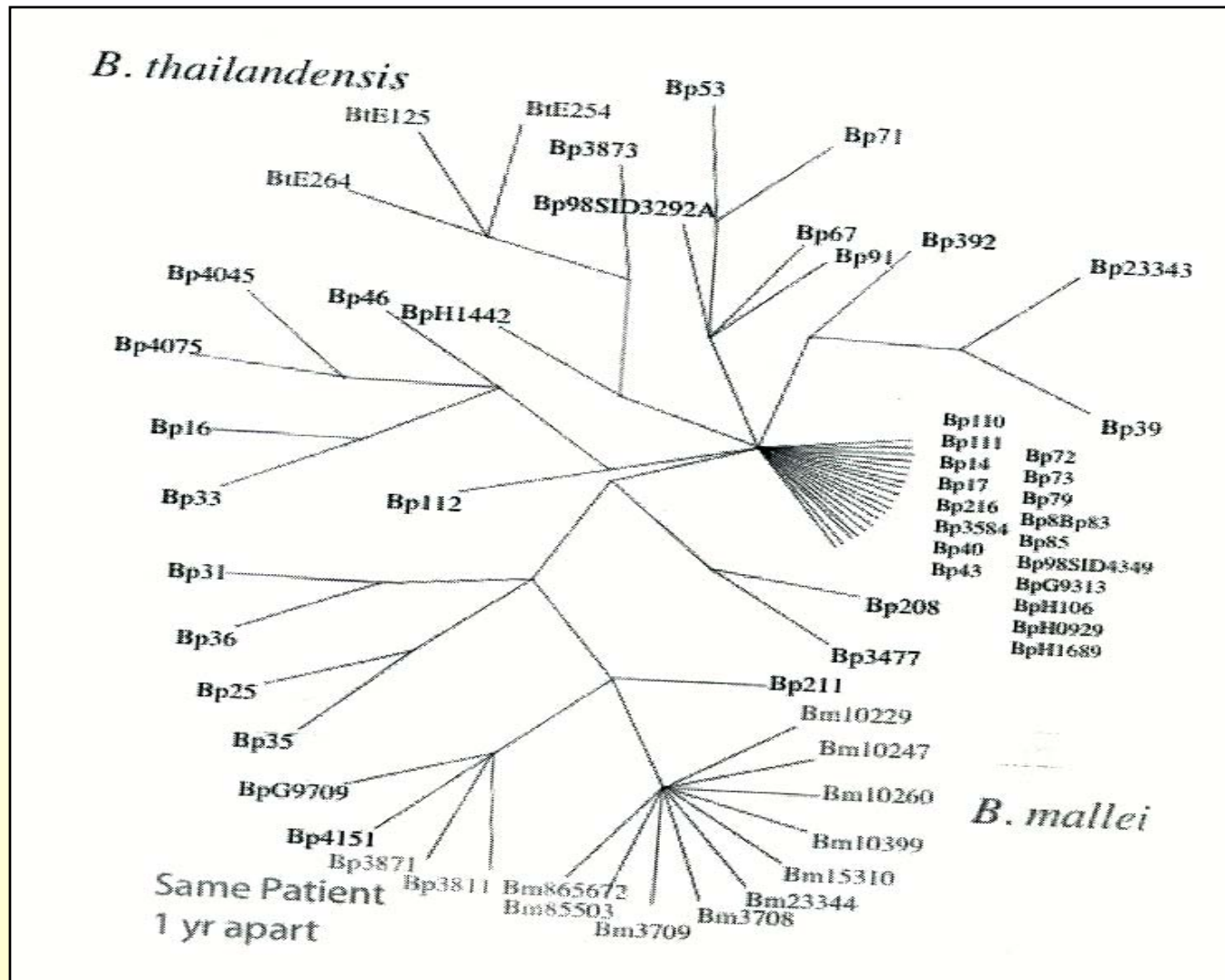
GOAL and APPROACH

- To develop real-time PCR assays, which are specific, both at the genus level (*Burkholderia*) and at the species level (*B. Mallei* and *B. pseudomallei*).
- DNA sequence analysis, loci targeting, and comparison with the Genbank database permitted identification of putative unique regions for design of primers and probe sets.

BACKGROUND

- *Burkholderia mallei* – causative agent for glanders mainly in animals – sporadically infects humans in Asia, Africa, the middle east, and South America.
- There is no vaccine and in case of infection, no dependable drug therapy is known.
- The DNA sequencing project completed (5.977×10^6 -bp).
- Among the gene sequences available – capsule gene cluster, 16S rRNA, IS, O-antigen biosynthetic gene cluster, flagellin, and few other genes.
- At present, no real-time PCR assays available either at the genus or at the species level.

Clustering of *Burkholderia* Strains



RESULTS AND DISCUSSION

- A total of 19 primer/probes designed so far.
 - ◆ 1 primer/probe set for detection at the genus level
 - ◆ 5 primer/probe sets for *B. pseudomallei*
 - ◆ 13 primer/probe sets for *B. mallei*
- Initial screening first performed by conventional PCR
- Primers/probe designed for genus level detection tested and confirmed through conventional PCR
- Only 5 sets of primers/probes (for species level detection) showed promise in the initial runs, and, therefore tested through RT-PCR assays
- RT-PCR assays performed with genomic DNA from one strain each of *B. mallei*, *B. pseudomallei*, *B. thailandensis*, and *P. diminuta*, using ABI-7900
- Assays for *B. pseudomallei* – ONLY 2 sets, BP9 & BP15 confirm specific signal amplification from test organism within 35 cycles
- Assays for *B. mallei* – ONLY 2 sets, BM6 and BM7 confirm specific signal amplification from test organism within 35 cycles

Selected Data – Genus Level

M1

8 7 6 5 4 3 2 1 H G F E D C B A P O N M L K J I

Genomic DNAs in 1 through 8 lanes were isolated from: 1 and 2 = *Burkholderia thailandensis*, E264 and E275, 3 = *Pseudomonas diminuta*; 4 = *P. syringae*; 5 = *Bacillus cereus*; 6 = *B. thuringensis*; 7 = *B. anthracis*; 8 = Sludge.

Genomic DNAs in A through H lanes were isolated from *Burkholderia pseudomallei*: A = E203; B = 316C; C = 1026b; D = Pateur52237; E = STW102-3; F = STW115-2; G = STW152; H = STW199-2.

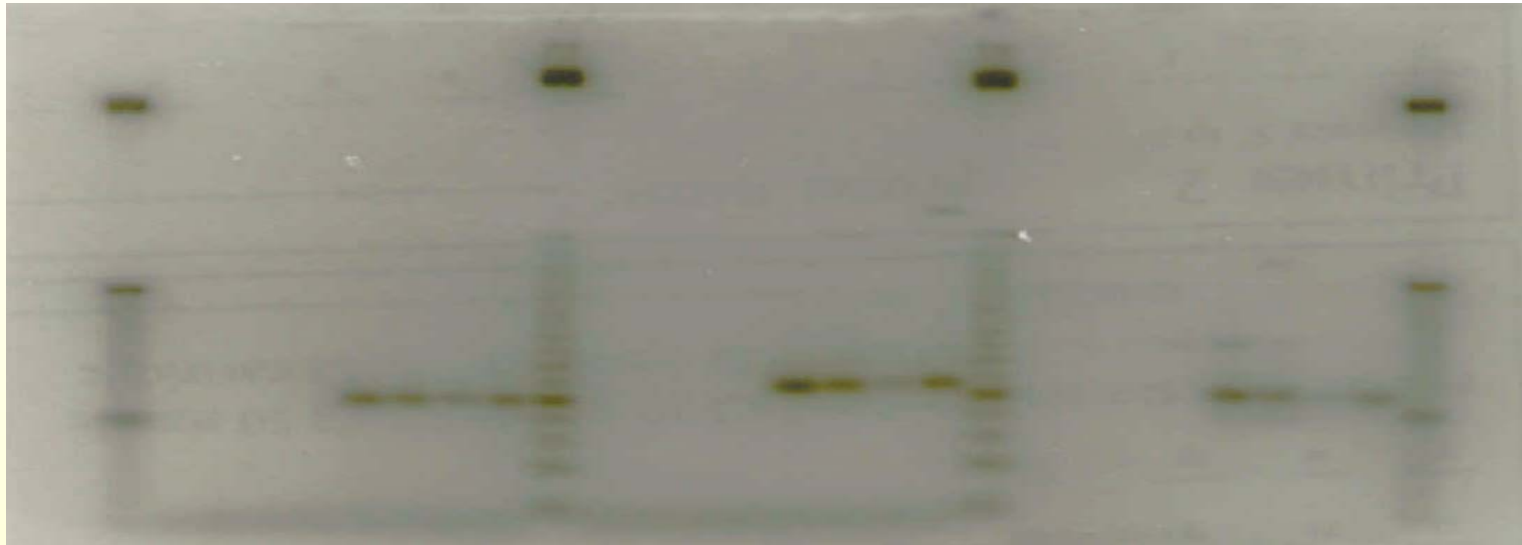
Genomic DNAs in I through P lanes were isolated from *Burkholderia mallei*: I = NCTC120; J = NCTC10229; K = NCTC10247; L = NCTC10248; M = NCTC10260; N = 10399; O = 15310; P = 23344.

M1 - 25-bp ladder; M2 -10-bp ladder.

Selected Data – Species Level

Burkholderia pseudomallei specific primers.

M1								M2								M2								M1															
								BP1								BP9								BP15															
1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8								



Genomic DNA from *B. mallei* and *B. pseudomallei*.

Lanes 1-4 = *B. mallei* NCTC (10260, 10399, 15310, 23344, respectively).

Lanes 5-8 = *B. pseudomallei* Pasteur52237 and STW (102-3, 115-2, and 152, respectively).

M1 – 10-bp ladder and M2 – 25-bp ladder (Invitrogen).

Selected Data – Species Level

Burkholderia mallei specific primers

M1								M2								M2								M1							
BM2								BM7								BM6															
1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8								



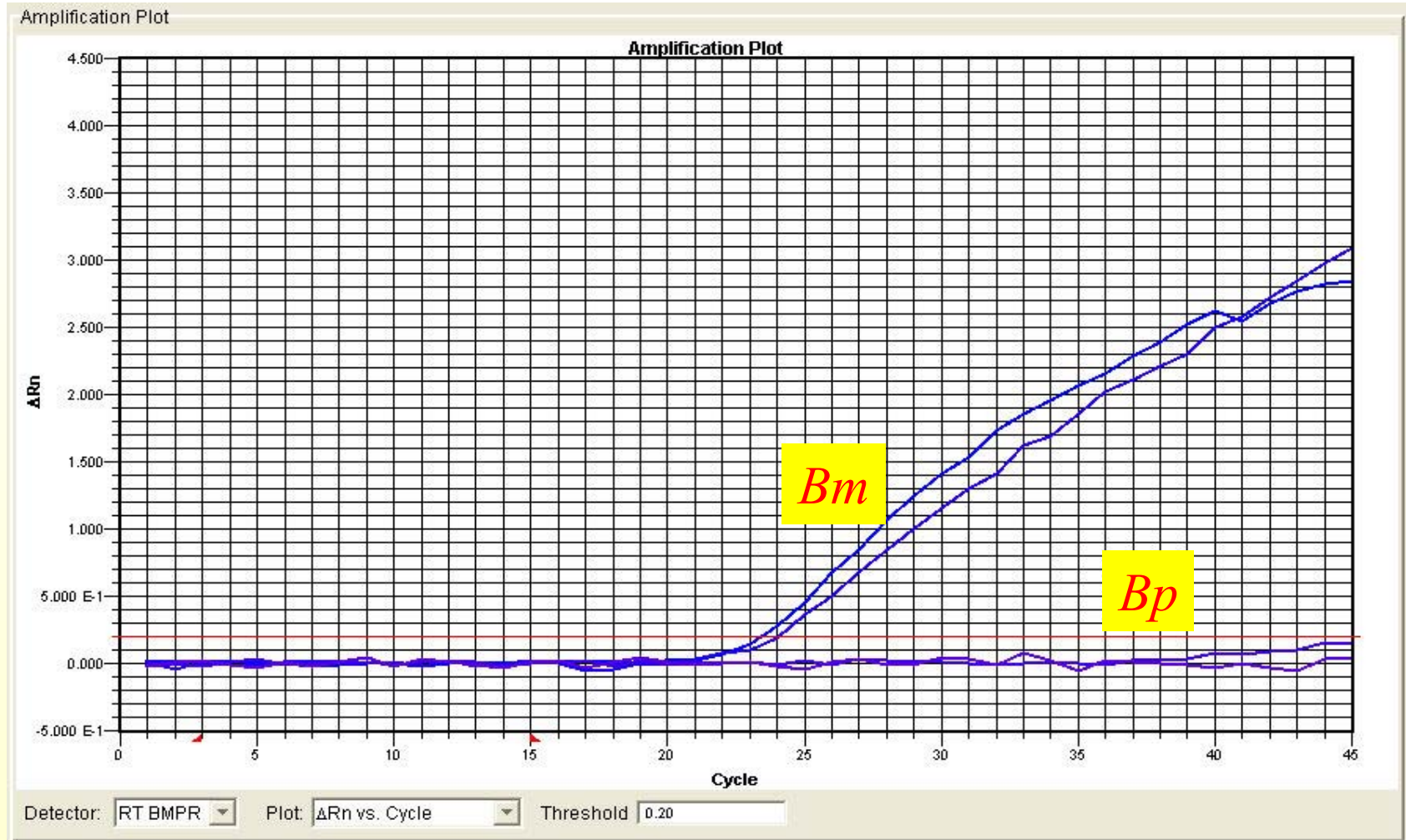
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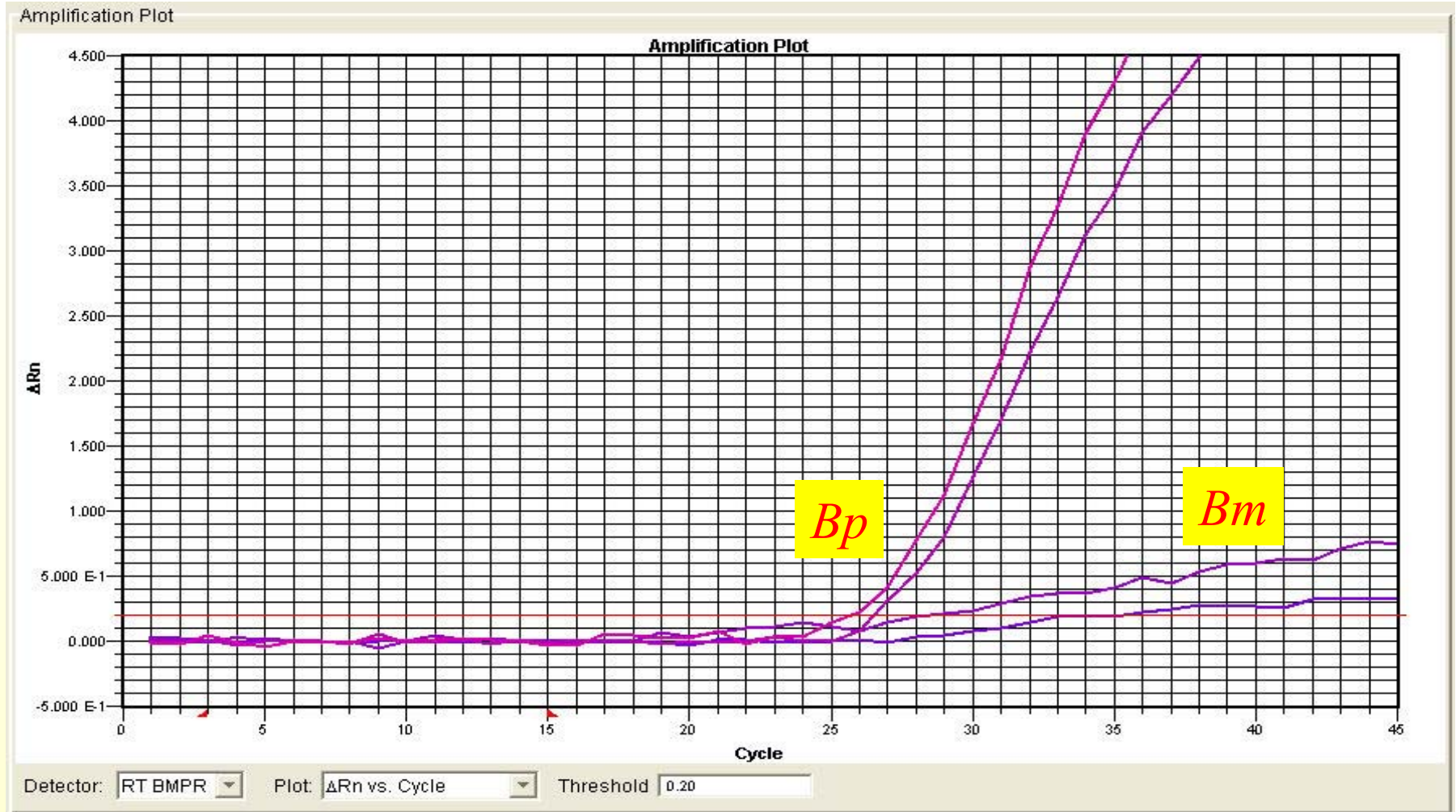
M1 – 10-bp ladder and M2 – 25-bp ladder (Invitrogen).

Specificity of *Burkholderia mallei* Primer Set and Probe # 6



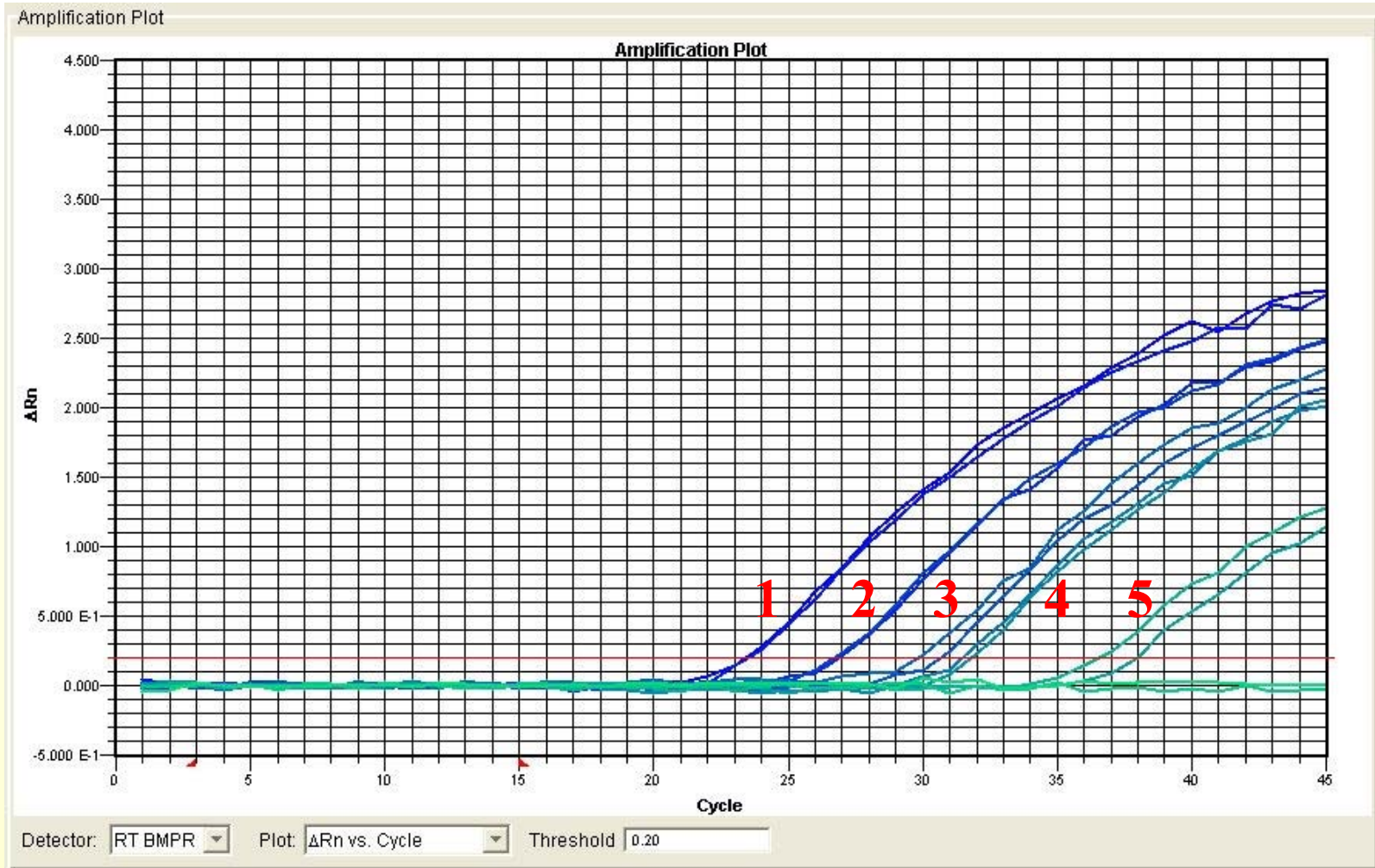
Bm = *Burkholderia mallei* and *Bp* = *Burkholderia pseudomallei*

Specificity of *Burkholderia pseudomallei* Primer Set and Probe # 9



Bm = *Burkholderia mallei* and *Bp* = *Burkholderia pseudomallei*

Titration of *Burkholderia mallei* DNA in RT-PCR Run using BM Primer Set and Probe # 6



1 = 100 pg; 2 = 10 pg; 3 = 100 fg; 4 = 50 fg; and 5 = 25 fg

CONCLUSIONS

- 1) Several sets of primers/probes have been identified designed and selected for PCR amplification with DNA from *B. mallei* and *B. pseudomallei*. The PCR amplified fragments have been analyzed with gel electrophoresis.
- 2) Specific detection at genus and species level for *B. mallei* and *B. pseudomallei* has been demonstrated with RT-PCR.
- 3) Similar approach can be extended for detection of other pathogens.

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