Efficacy Comparison of Various PCR Master Mixes and Thermal Cycling Parameters for Detection of *Brucella abortus* in Multiplex Assays

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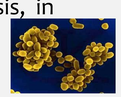
Abstract

Diagnostic testing for bioterrorism agents in food is essential for the safety and welfare of the general populace. Multiplex assays provide the means to test for multiple pathogen signatures with a single polymerase chain reaction (PCR) test. PCR, Luminex xMAP ® infrared fluorophore bead technology, and flow cytometry, multiplex diagnostic assays are incredibly specific and sensitive to highly conserved regions at various loci in a microbe's genome. In order to increase the effectiveness of the PCR and the resulting fluorescent response of the beads, different PCR master mix commercial kits were compared to the standard AgPath RT-PCR mix. This study specifically looks at *Brucella abortus* template 86/8/59 with the goal of finding the smallest concentration at which *Brucella abortus* can be detected and distinguished from *Brucella melitensis*.

What is *Brucella abortus*?

It is a gram-negative coccobacilli that infects cattle and can cause brucellosis; a zoonosis, in humans when infected meat is eaten.



What does brucellosis do in humans? In cattle?

In humans it causes acute undulating fever, headache, night sweats, fatigue and anorexia. In cattle, it causes abortions or prenatal defects. Brucellosis can be treated with antibiotics.

Why the need to detect and distinguish *Brucella abortus* and *Brucella melitensis*?

Like *B. abortus*, *B. melitensis* also causes brucellosis. It may infect sheep and cause late-term abortions or prenatal defects as well. Treatments and detection of these two species differ, so the need to accurately distinguish the two are needed. A fast and sensitive diagnostic test is essential to the cattle industry and for our own well-being.

PCR kits and Thermal Cycling Parameters

Commercial PCR kit	Thermal Cycling Parameters			
AgPath-ID™ One-Step RT-PCR Kit	Cycle	Repetitions	Temp.	Duration
	1	1	55°C	10:00
	2	1	95°C	2:00
	3	35	95°C	0:30
			60°C	0:30
			72°C	1:00
	4	1	72°C	2:00
	5	1	4°C	HOLD
Thermo Scientific Verso 1-Step RT-qPCR, Low ROX	Cycle	Repetitions	Temp.	Duration
	1	1	50°C	15:00
	2	40	95°C	0:15
			60°C	1:00
	3	1	4°C	HOLD
AccuStart™ Genotyping ToughMix®, Low ROX	Cycle	Repetitions	Temp.	Duration
	1	1	95°C	10:00
	2	40	95°C	0:15
			60°C	0:30
	3	1	4°C	HOLD
PerfeCTa® qPCR ToughMix®, Low ROX™	Cycle	Repetitions	Temp.	Duration
	1	1	95°C	3:00
	2	40	95°C	0:15
			60°C	0:30
	3	1	4°C	HOLD

Methods

Extract nucleic acids

Spike sample with alien arRNA. It is a unique RNA sequence within E. coli phage MS2 coat. It acts as the positive control to make sure the PCR worked.

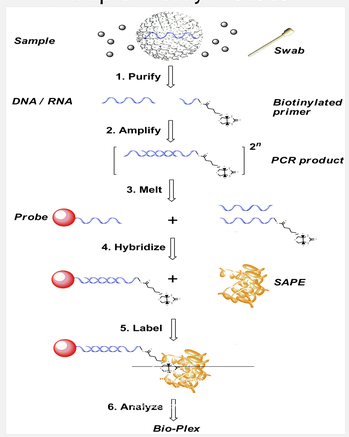
Amplify target sequences , multiplexed RT-PCR reaction using biotinylated primers

Hybridize biotinylated amplicons to target-specific probes on beads in multiplexed cocktail

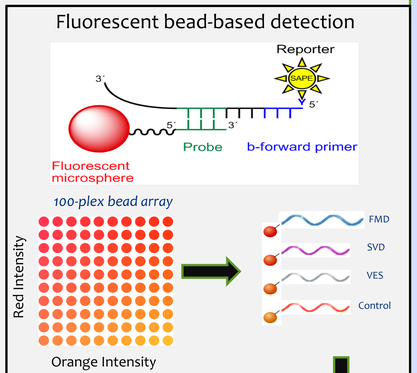
Detect hybridized amplicons with Streptavidin-R-phyco-erythryin fluorophore conjugate

Analyze in Bio-Plex reader

Multiplex Assay Protocol

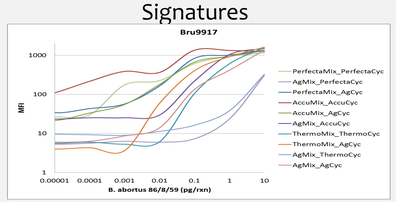


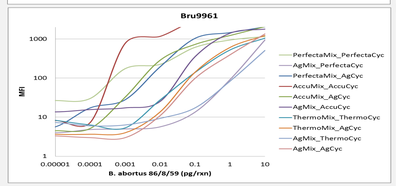
Fluorescent bead-based detection

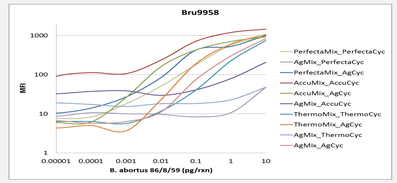


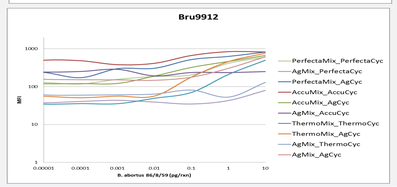
Results

Brucella abortus Chromosome 2 Signatures

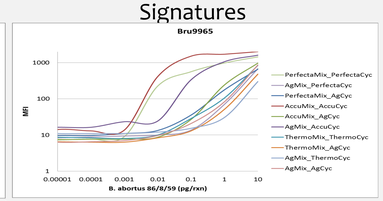


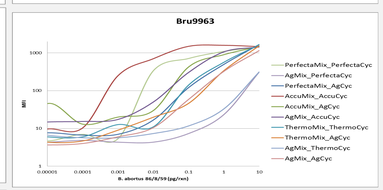




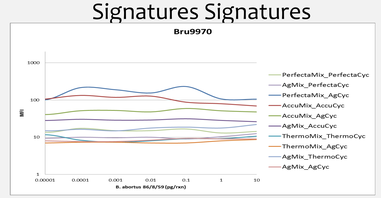


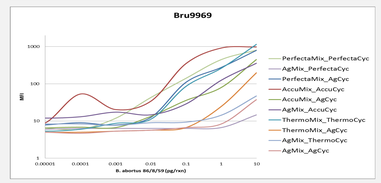
B. abortus Chromosome 1 Signatures



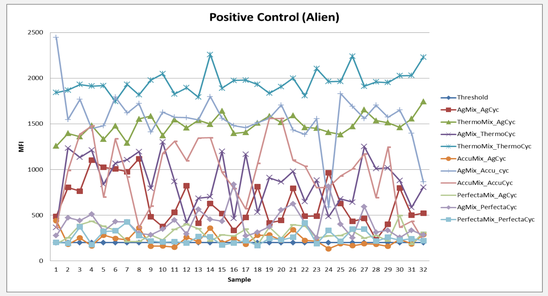


B. melitensis Chromosome 2 Signatures Signatures





Positive Control



Discussion

The Thermo and the AccuStart commercial kits, along with their respective thermal cyclings, were generally detecting the *B. abortus* at lower concentrations than the AgPath kit. The average MFI's, as shown by the positive control, were also much higher using these kits. The AgPath kit responded with much higher MFI using the thermal cyclings from the Thermo and AccuStart kits as well. The efficacy of the Thermo and AccuStart kits is much higher than the AgPath kit. If AgPath is continually going to be used, the thermal cyclings should be changed from it's set parameters to a more MFI-responsive set.

Future Studies

With adjustments to this multiplex panel (i.e. removing Bru9969 and Bru9912) and switching from the AgPath to the Thermo kit, more efficacy testing can be performed. Additional pathogen signatures can be added to the multiplex and a wider array of microbes can be detected for with this single multiplex assay as well.

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