Comparing the Accuracy of Two Diagnostic Tests, a 3-hour On-farm PCR System and Microbiological Culture, for Detecting the Presence of *Mycoplasma bovis* in Milk

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Introduction

MYCOB™ is a reagent manufactured by Acumen Detection Inc., Syracuse, NY that is used to determine the presence or absence in bovine milk of oligonucleotides from *Mycoplasma bovis* when used in the Acu-POLARIS™ 3-hour on-farm PCR system. This study used a robust number of samples of various concentrations of *M. bovis* spiked into known pathogen-free milk (based on complete extraction lab PCR) to refine the Limit of Detection (LOD), Limit of Quantification (LOQ), Sensitivity and Specificity of the test. The study also compared the PCR Sensitivity to the Sensitivity of microbiological culture.

Method

◀ Acu-POLARIS™ PCR

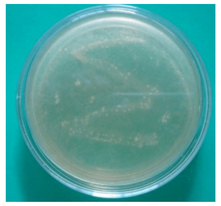
Sample Numbers for the 230 inoculated tubes were randomly assigned using a random number generator in Excel. Tubes were labeled with the random Tube Number. The Tube Number, Species of Inoculum, and Concentration of the Samples were entered into an Excel Master Sample Key file. The Sample Tubes were refrigerated and transferred to a second Lab Technician who was blinded as to sample type and concentration.

The blinded Lab Technician processed each sample using the prescribed methodology for the Acu-POLARIS system. Also, each unknown sample was streaked onto an appropriately labeled Mycoplasma Agar plate. The plates were incubated at 37°C under microaerophilic conditions (6% CO_2) and observed for the presence of Mycoplasma colonies at 3, 7, and 14 days.

Results

SAMPLES TESTED

1. Negative (Control) Sample = Known negative (pathogen-free) milk (50 vials)
2. Sample 1 = 1.1 x 10^2 CFU/mL *M. bovis* in milk (50 vials)
3. Sample 2 = 1.1 x 10^3 CFU/mL *M. bovis* in milk (50 vials)
4. Sample 3 = 1.1 x 10^4 CFU/mL *M. bovis* in milk (50 vials)
5. Sample 4 = 1.1 x 10^4 CFU/mL of *M. alkalescens* in milk (10 vials)
6. Sample 5 = 1.1 x 10^4 CFU/mL of *M. californicum* in milk (10 vials)
7. Sample 6 = 1.1 x 10^4 CFU/mL of *A. laidlawii* in milk (10 vials)



Results for the 3-hour PCR test and the microbiological culture were recorded on an Excel spreadsheet.

Positive PCR results (Ct≥10 and Ct<36 with ΔRFU≥115) were recorded as POSITIVES and all other results (Negative and Indeterminate results) were recorded as NEGATIVES. The visual presence of Mycoplasma colonies on the Mycoplasma Agar plates were recorded as POSITIVE cultures and the absence of any visual growth up to and including the 14-day reading were recorded as NEGATIVE cultures.

CULTURE RESULTS

Organism	Concentration	No. Pos. (Day 7)	Sensitivity	No. Pos. (Day 14)	Sensitivity
M. bovis	1.1 x 10^2 CFU/ml.	0/50	0%	0/50	0%
M. bovis	1.1 x 10^3 CFU/ml.	6/50	12%	6/50	12%
M. bovis	1.1 x 10^4 CFU/ml.	26/50	52%	34/50	68%

Acu-PCR™ RESULTS

Organism	Concentration	No. Positive	No. Negative	Sensitivity
Negative		0/50	50/50	0%
M. bovis	1.1 x 10^2 CFU/ml.	0/50	50/50	0%
M. bovis	1.1 x 10^3 CFU/ml.	47/50	3/50	94%
M. bovis	1.1 x 10^4 CFU/ml.	49/50	1/50	98%

Acu-PCR RESULTS

Organism	Concentration	No. Positive	No. Negative	Specificity
M. alkalescens	1.1 x 10^4 CFU/ml.	0/10	10/10	100%
M. californicum	1.1 x 10^4 CFU/ml.	0/10	10/10	100%
A. laidlawii	1.1 x 10^4 CFU/ml.	0/10	10/10	100%

Conclusion

In this laboratory study, the use of the MYCOB reagent in a 3-hour on-farm PCR system for detecting oligonucleotides associated with *Mycoplasma bovis* yielded the reagent's LOD as 1.1 x 10^3 CFU/mL and the reagent's LOQ as 1.1 x 10^4 CFU/mL. At the LOQ, the Sensitivity was 98% and the Specificity was 100%. By contrast, at this same concentration of *M. bovis*, the Sensitivity of microbiological culture was 52% when evaluated at 7 days of incubation and increased to 68% at 14 days of incubation. These results indicated that using the MYCOB reagent with a 3-hour on-farm PCR had a higher Sensitivity than microbiological culture and may be a useful tool when evaluating the presence of *M. bovis* oligonucleotides in milk samples.

References

Parker, Alysia M., et al. "A review of mycoplasma diagnostics in cattle." Journal of veterinary internal medicine 32.3 (2018): 1241-1252. 10.1111/jvim.15135.

Acknowledgments

RLS Management Solutions

Acumen DETECTION