RESEARCH SUMMARY:
ACUMEN MYCOB™ REAGENT

Roger Saltman, DVM, MBA, a consultant and former chief medical officer of U.S. cattle and equine business at Zoetis, has recently completed a study* demonstrating that on-farm milk testing with PCR technology that detects oligonucleotides (specific DNA fragments) of *Mycoplasma bovis* outperforms traditional culture testing done on farm or in a lab.

**Research results were presented at and published in the proceedings of the 2023 National Mastitis Council Annual Meeting in Atlanta, Ga.*

RESEARCH OVERVIEW

PURPOSE

- Refine the limit of detection (LOD), limit of quantification (LOQ), sensitivity and specificity of Acumen's MYCOB PCR reagent
- Compare the sensitivity of the Acumen MYCOB PCR Reagent to traditional microbiological culture in raw milk with specific concentrations of *Mycoplasma bovis (M. bovis)* added

OVERVIEW

The research study used a large number of raw milk samples with specific concentrations of the mastitis-causing pathogen *M. bovis* added. Each of the samples was processed using the Acu-POLARIS thermocycler/computer interface, Milk Lysis Prep Reagent, and MYCOB PCR reagent designed by Acumen Detection.

Microbiological cultures were evaluated at days 3, 7 and 14 to assure maximum growth. Since the study used known concentrations of *M. bovis* added to the milk, results from the Mycoplasma cultures could be compared to the PCR results, the percentage of true and false positives and true and false negative samples could be compared for each test at each specific concentration of *M. bovis*. The analysts did not know the sample concentration and the PCR was conducted once.

LAB PROCEDURE

- Samples were set up by Lab Technician A
- Samples were assigned a random number and logged into a master sample key file
- Lab Technician B received the unknown numbered samples and processed each sample using the Acu-POLARIS PCR procedure
- Each sample was also inoculated onto mycoplasma agar for culture analysis. After inoculation, the culture 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

RESEARCH RESULTS

MICROBIOLOGIC CULTURE RESULTS:

ORGANISM	CONCENTRATION	NUMBER OF POSITIVES (DAY 7)	SENSITIVITY	NUMBER OF POSITIVES (DAY 14)	SENSITIVITY
M. bovis	1.1×10^2 CFU/ml.	0/50	0	0	0
M. bovis	1.1×10^3 CFU/ml.	6/50	12%	6/50	12%
M. bovis	1.1×10^4 CFU/ml.	26/50	52%	34/50	68%

ACU-PCR RESULTS FOR DETECTION OF SPECIFIC OLIGONUCLEOTIDES:

ORGANISM	CONCENTRATION	NUMBER OF POSITIVES	NUMBER OF NEGATIVES	SENSITIVITY	SPECIFICITY
Negative	–	0	50	0%	–
M. bovis	1.1×10^2 CFU/ml.	0	50	0%	–
M. bovis	1.1×10^3 CFU/ml.	47/50	3/50	94%	–
M. bovis	1.1×10^4 CFU/ml.	49/50	1/50	98%	–
M. alkalescens	1.1×10^4 CFU/ml.	0/10	10/10	–	100%
M. californicum	1.1×10^4 CFU/ml.	0/10	10/10	–	100%
A. laidlawii	1.1×10^4 CFU/ml.	0/10	10/10	–	100%

SUMMARY:

The sensitivity of Acu-PCR using the MYCOB reagent is 98% and specificity is 100% for the detection of *Mycoplasma bovis* DNA oligonucleotides. At the same concentration, microbiologic culture yielded 52% sensitivity at 7 days of incubation and 68% sensitivity at 14 days of incubation. The specificity using three non-*M. bovis* related species (see table) was 100%, providing another advantage versus culture. Unlike PCR, microbiologic culture alone cannot distinguish *M. bovis* from *Acholeplasma* (which is not a pathogen) nor from other *Mycoplasma spp.* PCR results can be obtained in 3 hours vs. 7–14 days for microbiologic culture.