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Microbial identification of bovine milk isolates compared between conventional culture, MALDI-TOF and 16S rRNA

David J Wilson[1], **John R Middleton**[2], **Pamela R F Adkins**[2] and **Gregory M Goodell**[3]
[1]Utah State University, USA
[2]University of Missouri, USA
[3]The Dairy Authority LLC, USA

The objective of this study was to compare conventional microbial culture, MALDI-TOF (Matrix Assisted Laser Desorption Ionization - Time Of Flight) and 16S rRNA partial genomic sequencing methods for microbial identification in quarter milk samples from dairy cattle. The same microbial colonies were tested using each method. There is no agreed gold standard for true positive microbial identification. Therefore, this was a study of test agreement, not sensitivity or specificity; the latter calculations require true disease status. All 181 bacterial isolates were tested by culture and MALDI-TOF and 179 were tested by 16S rRNA because two isolates were lost during storage before the latter test was performed. For *Staphylococcus aureus* and *Escherichia coli* agreement was to the species level in accordance with conventional culture. For all other microbes, agreement was defined to the genus level or within the group defined as streptococcal like organisms in keeping with culture and accepted industry practices. All samples were mycoplasma negative. Overall agreement in identification of microbes between all three diagnostic methods was 94% (169/179). Agreement between MALDI-TOF and 16S rRNA was 98% (176/179); culture agreement with each of the other two methods was 95%. Specific microbes were identified with agreement among all three methods ranging from 97% to 100% all classified very good by the Kappa test. Many members of the dairy industry are used to either bacteriological culture or MALDI-TOF for routine mastitis pathogen diagnosis and there is interest in the agreement between the methods. These results suggest that either method is of practical value. At present 16S rRNA testing is primarily a research tool but it showed high agreement with the other methods. For purposes of milk quality and udder health monitoring any of the three methods are valuable tools for the dairy industry.

David.Wilson@usu.edu