

The Use of Modules for the Detection of Pathogens in Mastitic Milk and *Mycobacterium avium* subsp. *Paratuberculosis* in Bovine Feces with the MagMAX™ CORE Nucleic Acid Purification Kit

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ABSTRACT

In many testing labs, the kit used to isolate nucleic acids from pathogens depends largely on the sample type and pathogen of interest. The need to use multiple extraction kits reduces efficiency and adds complexity to testing. For example, the product used to isolate nucleic acid from *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in bovine feces is different than the product used for mastitis testing in milk, which in turn is different than the product required for Bovine Viral Diarrhea Virus (BVDV) from ear notches. The MagMAX™ CORE Nucleic Acid Purification Kit allows for testing numerous sample types with a single kit that uses a core workflow for all sample types. This provides a huge benefit for lab efficiency and simplifies testing by allowing multiple sample types to be run at the same time. All MagMAX CORE reagents were designed to be stored at room temperature, the wash solutions are pre-mixed eliminating the need to purchase additional isopropanol and ethanol, and the number of washes has been reduced. The kit was optimized for many sample types including BVDV from ear notches with the intent of future modules to be developed that would meet additional testing needs. Here, we show the extraction of MAP DNA from bovine feces with the new MagMAX CORE Mechanical Lysis Module performs equivalently or better than two currently available nucleic acid purification kits which were specifically designed for the detection of MAP. Additionally, we show extraction of numerous pathogens from milk known to cause mastitis using the MagMAX CORE Mastitis and Panbacteria Module. The results show equivalent or better recovery for all pathogens tested. Combined, these studies shows that the MagMAX CORE Nucleic Acid Purification Kit is a universal sample preparation kit that will meet all future nucleic acid purification needs.

INTRODUCTION

The MagMAX CORE Nucleic Acid Purification Kit was designed to meet or exceed the performance of currently available magnetic bead based kits on the market while improving user experience. In many labs, different extraction kits are required depending on both the sample type and the nucleic acid type. The MagMAX CORE Nucleic Acid Purification Kit has been validated for use with many animal matrices for both DNA and RNA applications. Table 1 lists 14 matrices tested with the MagMAX CORE Nucleic Acid Purification Kit. In order to address sample types not listed in Table 1, modules that build upon the MagMAX CORE Nucleic Acid Purification Kit will be developed to address additional testing needs. The MagMAX CORE Mechanical Lysis Module was specifically designed to extract DNA from difficult to-lyse bacteria such as MAP. The MagMAX CORE Mastitis and Panbacteria Module was designed to lyse and isolate the wide variety of difficult to lyse bacteria known to cause mastitis in bovine milk. These two modules are examples of how modules will be developed to increase the flexibility of the MagMAX CORE Nucleic Acid Purification Kit.

MATERIALS AND METHODS

For MAP detection, eight positive and 3 negative field bovine fecal samples were obtained. The fecal samples were tested with both 0.3g and 5g inputs using the MagMAX CORE Mechanical Lysis Module. The 0.3g samples were compared to a commercial kit designed for MAP detection using 0.3g of feces. The 5g input was compared to a separate commercial kit designed to use 2-5g of fecal input. The KingFisher™ Flex Purification System was used for all isolations. The VetMAX™-Gold MAP Detection Kit from Applied Biosystems™ was used for real-time detection to compare extraction of MAP DNA.

For mastitis detection, 20 mastitic milk samples were obtained with 20 different targets known to cause mastitis in cattle. These included multiple gram positive and gram negative bacterial species, as well as Prototheca and yeast. The MagMAX CORE Mastitis and Panbacteria Module was used with 200ul of milk. This was compared to a commercial purification kit design for mastitis pathogen extraction from milk. A commercially available PCR kit for the detection of mastitis was used for the real-time PCR detection of the 20 different DNA targets.

Table 1. Sample types validated with MagMAX CORE Nucleic Acid Purification kit

• Whole blood	• Semen
• Feces	• Serum
• Oral fluid	• Ear notches
• Animal, fecal, and environmental swabs	• Ear punches
• Tissue / Organs	• Environmental samples
• Milk	• Biomed Diagnostics InPouch™ TF (Tritrichomonas foetus) culture
• Hair follicles	• Plasma

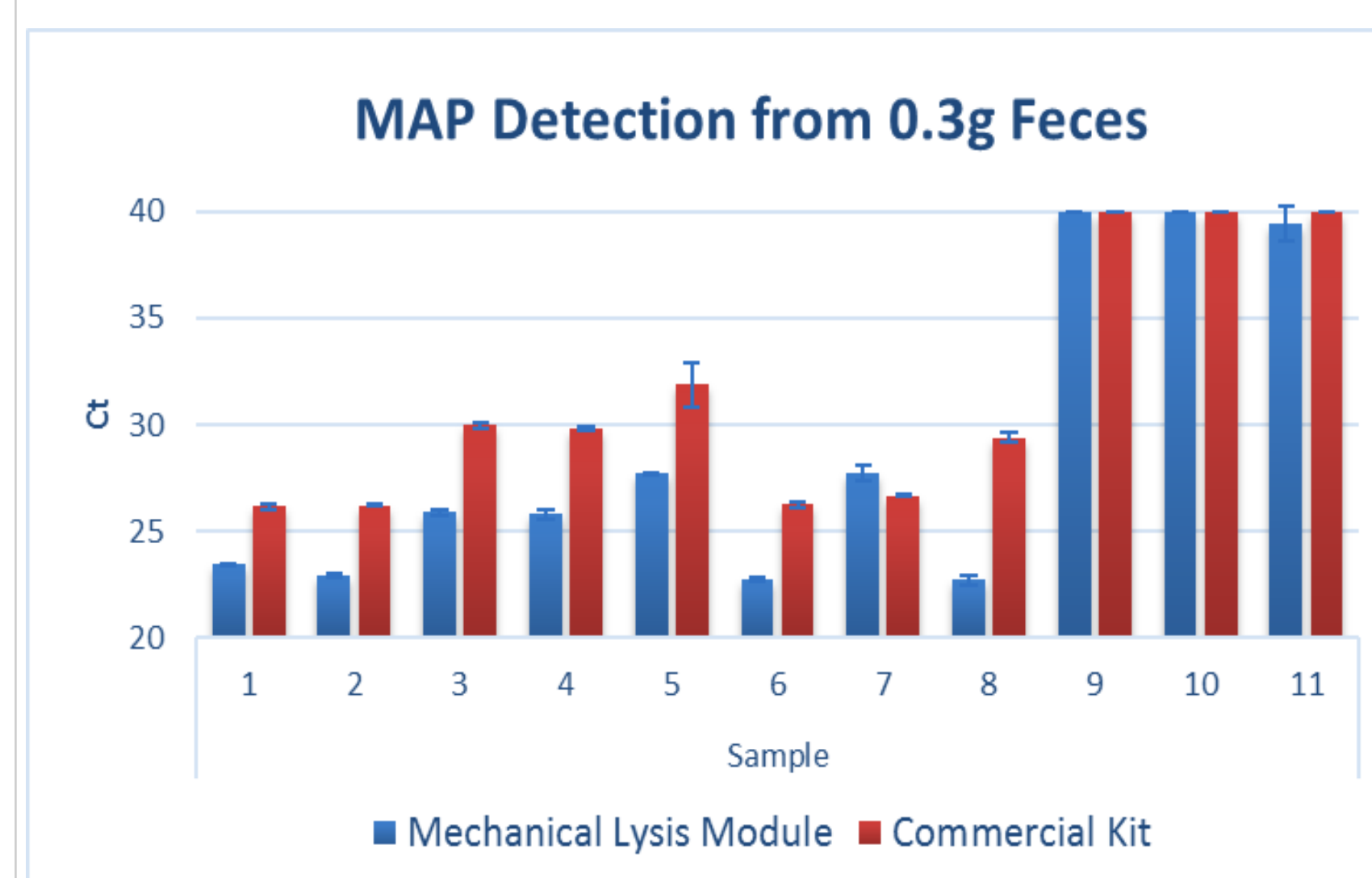
RESULTS

Figure 1. MagMAX CORE Mechanical Lysis Module



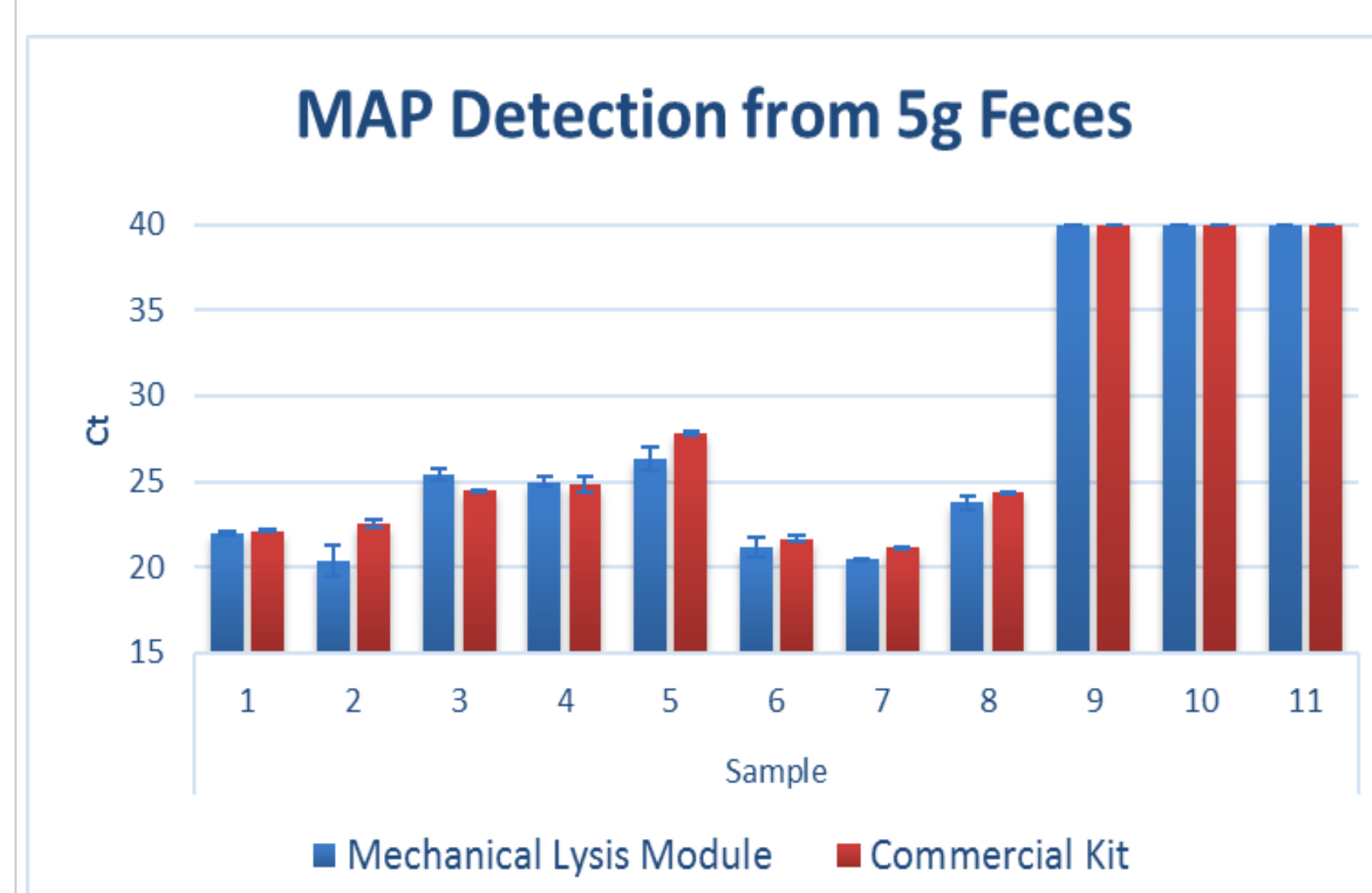
The MagMAX CORE Mechanical Lysis Module comes complete with pre-filled bead beating tubes. The bead tubes, together with a specially optimized Clarifying Solution, provide a highly efficient method of physical disruption of mycobacteria known to cause MAP.

Figure 2. MAP Detection Using a Low Input of Bovine Feces



8 positive and 3 negative bovine fecal samples were tested with 0.3g of input with the MagMAX CORE Mechanical Lysis Module. This was compared to a commercially available kit that also uses 0.3g of input for MAP detection. The VetMAX™-Gold MAP Detection Kit from Applied Biosystems™ was used for real-time detection to compare extraction of MAP DNA. On average, the MagMAX CORE Mechanical Lysis Module yielded a Ct that was 3.4 ± 2.2 better than the commercially available kit for MAP DNA isolation.

Figure 3. MAP Detection Using a High Input of Bovine Feces



8 positive and 3 negative bovine fecal samples were tested with 5g of input with the MagMAX CORE Mechanical Lysis Module. This was compared to a commercially available kit that uses 2-5g of input for MAP detection. The VetMAX™-Gold MAP Detection Kit from Applied Biosystems™ was used for real-time detection to compare extraction of MAP DNA. The MagMAX CORE Mechanical Lysis Module and the commercial kit showed no statistical difference. The two kits resulted in a 0.6 ± 1.0 Ct difference in favor of the MagMAX CORE Mechanical Lysis Module.

Figure 4. MagMAX CORE Mastitis and Panbacteria Module



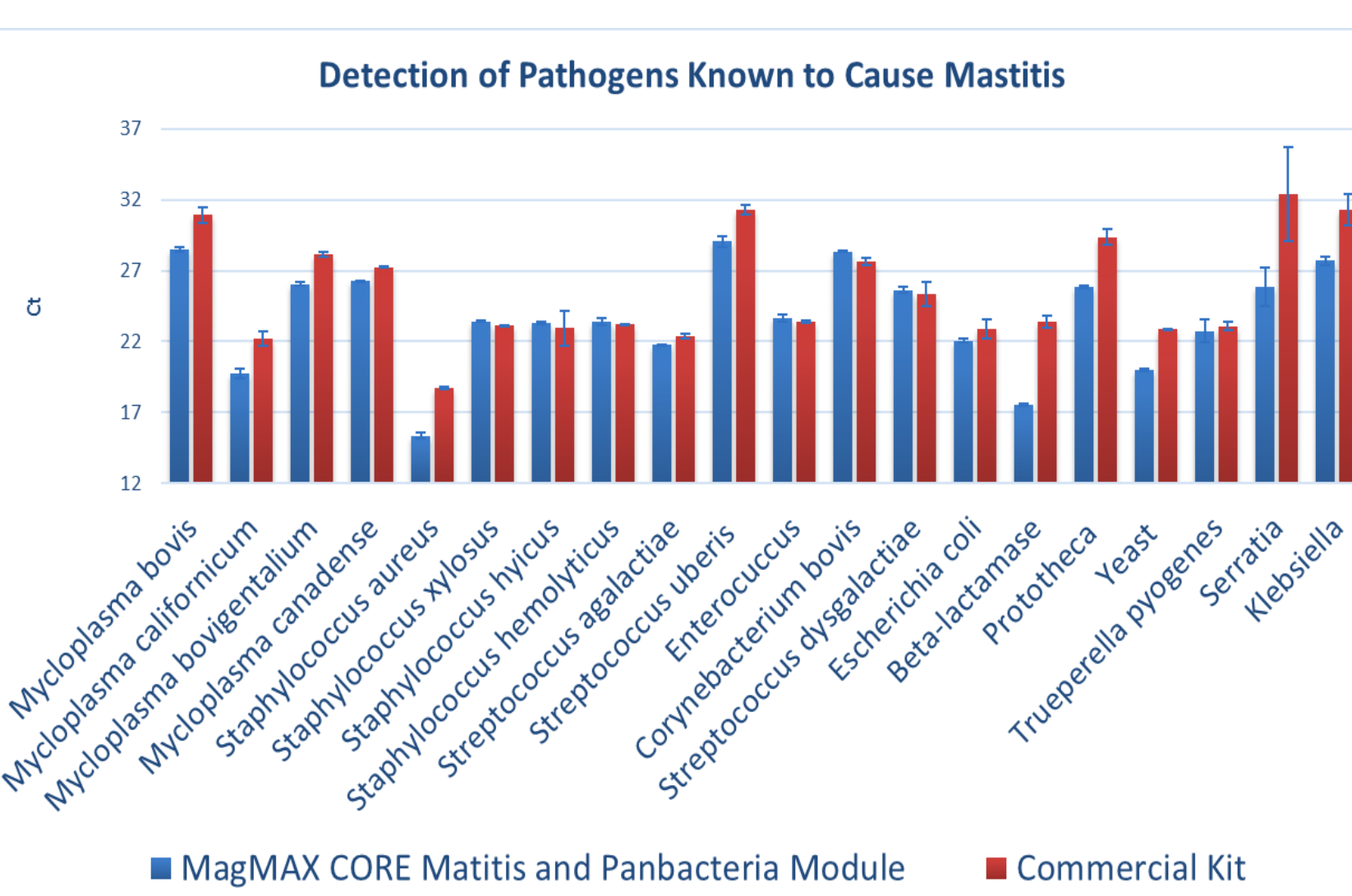
The MagMAX CORE Mastitis and Panbacteria Module is shown here with the 100 reaction MagMAX CORE Nucleic Acid Purification Kit. The solution supplied with the MagMAX CORE Mastitis and Panbacteria Module is specially designed to help isolate DNA from a wide range of pathogen targets including both gram positive and gram negative bacteria.

Figure 5. MagMAX CORE Mastitis and Panbacteria Workflow Comparison

MagMAX CORE	Supplier C
Add lysis solution to milk sample	Add milk sample to Solution 1 and incubate for 10 min at 37°C
Mix for 5 min at room temp	Centrifuge for 5 min at 37°C
Add 10 µL Proteinase K	Aspirate supernatant
Load plates on KingFisher Flex system	Add wash buffer
Add 720 µL of bead mix	Centrifuge for 5 min at 37°C
Retrieve sample for PCR	Aspirate supernatant
	Add 80 µL of Lysis-1 Mix
	Vortex
	Spin for 20 sec
	Transfer to 96-well plate
	Incubate at 37°C for 20 min
	Incubate at 95°C for 15 min
	Cool on ice for 5 min
	Centrifuge for 5 min
	Transfer to 96-well plates
	Start PCR reaction

The workflows for the MagMAX CORE Mastitis and Panbacteria Module is shown compared to a commercially available kit designed for mastitis DNA isolation. The workflow for the MagMAX CORE Mastitis and Panbacteria Module is significantly simpler than other workflows designed for mastitis DNA isolation from milk. All centrifugation and aspiration steps have been removed from the workflow. This saves a significant amount of time while reducing the possibility of sample cross contamination.

Figure 6. MagMAX CORE Mastitis and Panbacteria Pathogen Detection Comparison



20 mastitic milk samples were obtained with 20 different targets known to cause mastitis in cattle and tested with the MagMAX CORE Mastitis and Panbacteria Module. This was compared to a commercial purification kit design for mastitis pathogen extraction from milk. A commercially available PCR kit for the detection of mastitis was used for the real-time PCR detection of the 20 different DNA targets. For 8 of the targets there is no statistical difference between the two extraction kits. For the other 12 targets, the MagMAX CORE module yields a Ct that is 3.06 ± 1.71 better than the commercial kit.

CONCLUSIONS

The MagMAX CORE Mechanical Lysis Module offers a complete solution for the isolation of MAP DNA from bovine fecal samples. The bead beating tubes allow for a wide range of inputs that make the module flexible and easily adaptable to labs currently testing for MAP. Figure 2. shows with a low input of 0.3g, the MagMAX CORE Mechanical Lysis Module yielded a Ct that was 3.4 ± 2.2 better than the commercially available kit for MAP DNA isolation. Figure 3. shows the results when comparing to a different commercially available kit that uses a higher input of bovine feces. No statistical difference was observed, but the results showed a trend of better Ct values for the MagMAX CORE Mechanical Lysis method. Together, this shows that the MagMAX CORE Mechanical Lysis Module offers a highly flexible workflow that can accommodate a low or high input of bovine feces.

Figure 5. shows the workflow of the MagMAX CORE Mastitis and Panbacteria Module compared to another commercially available kit designed for mastitis DNA isolation. The MagMAX CORE Mastitis and Panbacteria Module has a significantly simpler workflow with all centrifugation and aspiration steps eliminated from the workflow. This saves a significant amount of time while reducing the possibility of sample cross contamination. Figure 6. shows the results from 20 mastitic milk samples with 20 different targets known to cause mastitis in cattle. The MagMAX CORE Mastitis and Panbacteria Module was compared to a commercial purification kit design for mastitis pathogen extraction from milk. 8 of the targets showed no significant difference in isolation. In the other 12 targets, the MagMAX CORE Mastitis and Panbacteria Module yields a Ct that is 3.06 ± 1.71 better than the other commercial kit. This shows that though the MagMAX CORE Mastitis and Panbacteria Module has a much simplified workflow, it yields generally better results than the other commercial kit.

Together, the results from these two modules shows that the MagMAX CORE Nucleic Acid Purification Kit offers additional usability and flexibility to the kit. Future modules will be developed to address new samples matrices that may come up in the future that will use the same CORE chemistry.

Ordering Information

Product	Unit Size	Catalog Number
MagMAX CORE Mechanical Lysis Module	100 tests	A32836
MagMAX CORE Mastitis and Panbacteria Module	100 tests	A40289
MagMAX CORE Nucleic Acid Purification Kit	100 tests	A32700
MagMAX CORE Nucleic Acid Purification Kit	500 tests	A32702

TRADEMARKS/LICENSING

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