

Mastitis Diagnosis utilizing a turnkey solution from Thermo Fisher Scientific's Animal Health Group



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INTRODUCTION

Bovine mastitis is the most common and costly disease among dairy cows. It is an inflammation of the mammary gland that is predominantly caused by a bacterial infection. When mastitis is identified in a cow's quarter(s), it is important to identify the pathogen causing the infection because different categories of pathogens require different management strategies. Once the infection is identified, a dairy farmer can work with his or her veterinarian to target an effective treatment plan. Prudent use of antibiotics reduces the likelihood of resistant pathogens developing and can reduce the duration of treatment a cow may need, which in turn decreases operating costs.

Applied Biosystems™ VetMAX™ MastiType multiplex qPCR kits, together with the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit, are designed to provide same-day results even for *Mycoplasma* spp., enabling farmers and veterinarians to take fast and well informed action. It provides a rapid workflow that allows diagnosis of milk samples for the presence of 19 different types of microorganisms and penicillin-resistance genes. The extracted DNA can then be tested with three different 4-plexed VetMAX™ MastiType PCR kits, on either the Applied Biosystems™ 7500 or QuantStudio™ 5 series of Real-Time PCR Systems. Each DNA sample is analyzed in a single-well: the same well is used to specifically detect the DNAs of four pathogens and an Internal Amplification Control (IAC). A positive IAC means both successful extraction and the absence of PCR inhibitors in the sample. Data analysis for each of these uses a new cloud-based software with a user-friendly graphical interface.

Nucleic Acid extraction + MagMAX CORE Nucleic Acid Purification Kit + MagMAX CORE Mastitis & Panbacteria Module

Ref. A40289

- 1 x 50 mL MagMAX CORE Lysis Solution*
- 1 x 45 mL MagMAX CORE Binding Solution*
- 1 x 60 mL MagMAX CORE Wash Solution 1*
- 1 x 60 mL MagMAX CORE Wash Solution 2*
- 1 x 12 mL MagMAX CORE Elution Buffer*
- 1 x 2.2 mL MagMAX CORE Magnetic Beads*
- 1 x 1.25 mL MagMAX CORE Proteinase K*
- 4 x 1375 µL MagMAX CORE Mastitis PanBacteria#

* Store at room temperature.
Store at -5 to -30°C.

Milk Samples

- ✓ Bulk milk
- ✓ Preserved milk* (e.g., with bronopol)
- ✓ Frozen milk
- ✓ Mastitic milk
- ✓ Milk with high SCC



*Use of preserved milk samples eliminates the need for cooled transport of samples

Protocol

200µL of Milk + 50µL MagMAX CORE Mastitis Panbacteria Solution
Mix for 5 min at room temperature
Add 10µL MagMAX CORE Proteinase K

Manual extraction



DynaMag-2 Magnets
16 samples

Automated extraction



KingFisher Duo Prime
24 samples



KingFisher Flex/96
96 samples

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Turn on KingFisher load script

Start Run and load 4 plates (2 washes)



10min



Add Lysis/Binding Beads
Load the sample plate back



Press Start to continue



+27min

Nucleic Acid

Amplification

VetMAX MastiType Kits

Micro4

Real-time PCR detection of 4 mastitis-causing pathogens in a single PCR reaction



- Master Mix
- Positive Control
- Micro4 Primer Mix

Catalogue number A39235

Pathogens identified

- *Mycoplasma bovis*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Streptococcus uberis*

Myc08

Real-time PCR detection of 8 mastitis-causing pathogens in two separate PCR reactions



- Master Mix
- Positive Control
- Myco8 Primer Mix 1
- Myco8 Primer Mix 2

Catalogue number A39236

Pathogens identified

- *Mycoplasma* spp.
- *Mycoplasma alkalescens*
- *Mycoplasma bovis*
- *Mycoplasma bovigenitalium*
- *Mycoplasma canadense*
- *Mycoplasma californicum*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*

Multi

Real-time PCR detection of mastitis-causing pathogens and β-lactamase gene in four separate PCR reactions



- Master Mix
- Positive Control
- Multi Primer Mix 1
- Multi Primer Mix 2
- Multi Primer Mix 3
- Multi Primer Mix 4

Catalogue number A39227

Pathogens identified

- *Staphylococcus aureus*
- *Staphylococcus* spp.
- *Streptococcus agalactiae*
- *Streptococcus dysgalactiae*
- *Streptococcus uberis*
- *Escherichia coli*
- *Enterococcus* spp.
- *Klebsiella oxytoca* and/or *K. pneumoniae*
- *Serratia marcescens*
- *Corynebacterium bovis*
- *Trueperella pyogenes*
- *and/or Paenibacillus indolicus*
- *Staphylococcal β-lactamase Gene (penicillin-resistant gene)*
- *Mycoplasma bovis*
- *Mycoplasma* spp.
- *Yeast*
- *Prototheca* spp.

Amplification

PCR Reaction Mix

For 1 well
• 10µL MastiType Master Mix
• 5µL MastiType Primer Mix

For 1 well
• 15µL PCR Reaction Mix

PCR Reaction Plate

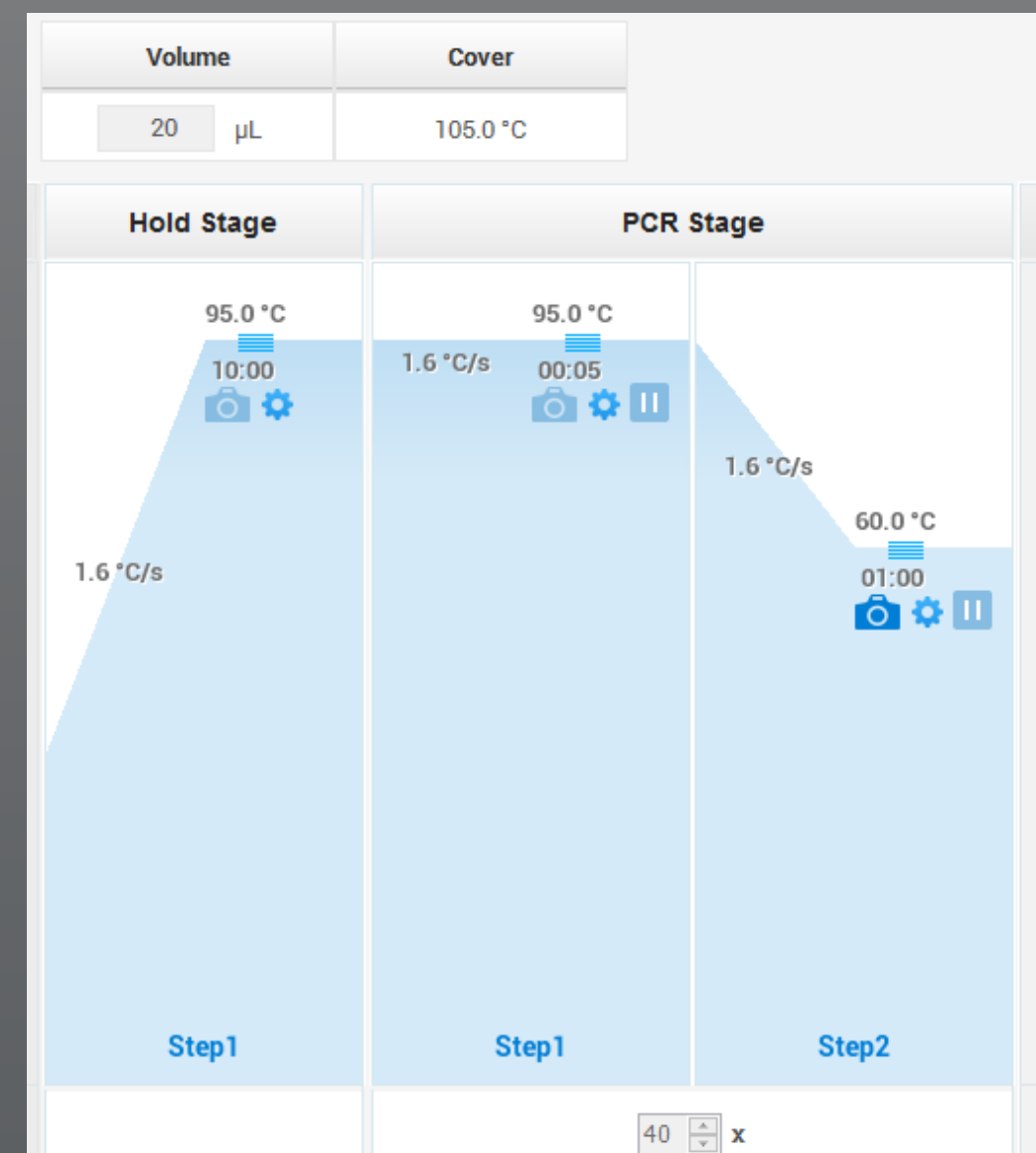
For 1 well
• 5µL Sample type

Close the plate with optically clear caps

Ultra Clear qPCR Caps, strips of 8

Catalogue number: A80666

Set up, then run the real-time PCR



Run complete EDS file

Animal Health VeriVet Software

For free on Thermo Fisher Cloud
Sign in to thermofisher.com/cloud using Thermo Fisher account

- Cloud for data analysis and storage
- Use with 7500 and Q55
- Fully automated analysis
- Easy readout of POS and NEG results



7500 real-time PCR system QuantStudio 5 real-time PCR system

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Interpretation

VeriVet Software

Display plots and well details Select Assays Select View

1 2 3 4 5

Ct call buttons

- Pathogen DNA is detected (below cut off)
- Pathogen DNA is not detected
- Pathogen DNA is detected in quantity above the assay's cut-off value
- Internal Amplification Control (IAC) failed and pathogen DNA is not detected

Amplification plots

Export result (Excel format)

Well	Result	Sample	Assay	Target	Ct	Target Result	Custom Call
A1	NEGATIVE		VetMAX MastiType Multi 1	M. bovis	0.00	NEGATIVE	
				IAC	29.55	POSITIVE	
				S.aureus	0.00	NEGATIVE	
A2	POSITIVE		VetMAX MastiType Multi 2	Enterococcus spp.	0.00	NEGATIVE	
				C. bovis	0.00	NEGATIVE	
				Str. dysgalactiae	27.98	POSITIVE	++
				beta-lactamase	0.00	NEGATIVE	
				IAC	26.93	POSITIVE	
				Mycoplasma spp.	0.00	NEGATIVE	
A3	POSITIVE		VetMAX MastiType Multi 3	E. coli	0.00	NEGATIVE	
				Str. Agalactiae	0.00	NEGATIVE	
				Str. Uberis	0.00	NEGATIVE	
				Prototheca spp.	0.00	NEGATIVE	
				Staph. Spp.	32.82	POSITIVE	+
				IAC	23.74	POSITIVE	
A4	POSITIVE		VetMAX MastiType Multi 4	T.pyo-Pstr.indolicus	0.00	NEGATIVE	
				Yeast	33.52	POSITIVE	+
				Serratia marcescens	0.00	NEGATIVE	
				IAC	20.38	POSITIVE	
				Klebsiella spp.	0.00	NEGATIVE	
				M. bovis	0.00	NEGATIVE	
A5	POSITIVE		VetMAX MastiType Multi 1	IAC	28.65	POSITIVE	
				S.aureus	35.00	POSITIVE	+
				Enterococcus spp.	22.35	POSITIVE	+++
				C. bovis	0.00	NEGATIVE	
				Str. dysgalactiae	20.83	POSITIVE	+++
				beta-lactamase	30.02	POSITIVE	+
A6	POSITIVE		VetMAX MastiType Multi 2	IAC	26.57	POSITIVE	
				Mycoplasma spp.	0.00	NEGATIVE	
				E. coli	34.16	POSITIVE	+
				Str. Agalactiae	0.00	NEGATIVE	
				Str. Uberis	37.64	SUSPECT	
				Prototheca spp.	0.00	NEGATIVE	
A7	POSITIVE		VetMAX MastiType Multi 3	Staph. Spp.	28.51	POSITIVE	++
				IAC	23.39	POSITIVE	
				T.pyo-Pstr.indolicus	0.00	NEGATIVE	
				Yeast	0.00	NEGATIVE	
				Serratia marcescens	0.00	NEGATIVE	
				IAC	20.01	POSITIVE	
A8	NEGATIVE		VetMAX MastiType Multi 4	Klebsiella spp.	0.00	NEGATIVE	
				IAC	20.01	POSITIVE	

Interpretation

Target result	Custom call	Interpretation
Negative	-	Pathogen DNA is not detected
Positive	+	Pathogen DNA is detected in low quantity
Positive	++	Pathogen DNA is detected in intermediate quantity
Positive	+++	Pathogen DNA is detected in high quantity
Suspect	-	Pathogen DNA is detected in quantity above the assay's cut-off value
Inconclusive	-	IAC failed and pathogen DNA is not detected

Note: Ct values >37, but <40 can indicate that target is present at low level. We recommend that ct values >37 are considered negative.

RESULTS

Characteristics of complete method		
Number of target tested on milk field samples	Positive	289
	Negative	1059
Similar results (%) MastiType workflow compared to another workflow	> 95%	

CONCLUSION

The MastiType workflow takes about 3 hours. The MagMAX CORE Nucleic Acid Purification Kit has been validated on mastitic milk field samples containing each of the target organisms. DNA mastitis samples have been validated on the 3 different 4-plexed kits called VetMAX MastiType Kit and provides equivalent results on the 7500 and QuantStudio 5 Real-Time PCR Systems. The Thermo Fisher Scientific mastitis solution is a diagnostic tool that is easy to handle and can provide rapid and accurate results in order to help ensure the efficacy of surveillance and control programs.

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