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 infective 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 BiosystemsTM VetMAXTM MastiType multiplex qPCR kits, together with the Applied BiosystemsTM MagMAXTM 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 VetMAXTM 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.

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• Staphylococcal β-lactamase

Gene (penicillin-resistant gene)





NUCIEIC ACIA extraction

+ MagMAX CORE Mastitis & Panbacteria Module



• 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

200uL of Milk + 50uL MagMAX CORE Mastitis Panbacteria Solution

Mix for 5 min at room temperature

Add 10ul MagMAX CORE Proteinase K

Amplification	VetMAX MastiType Kits			
Micro4	Мусо8	Multi		
Real-time PCR detection of 4 mastitis-causing pathogens in a single PCR reaction	Real-time PCR detection of 8 mastitis-causing pathogens in two separate PCR reaction	Real-time PCR determastitis-causing patho β-lactamase gene separate PCR rea		
Image: State State State	Applied OSSISTEMS THE REAL THE RE			
 Master Mix Positive Control Micro4 Primer Mix Catalogue number A39235 	 Master Mix Positive Control Myco8 Primer Mix 1 Myco8 Primer Mix 2 Catalogue number A39236	 Master Mix Positive Control Multi Primer Mix 1 Multi Primer Mix 2 Multi Primer Mix 3 Multi Primer Mix 4 		
 Pathogens identified Mycoplasma bovis Staphylococcus aureus Streptococcus agalactiae Streptococcus uberis 	 Pathogens identified Mycoplasma spp. Mycoplasma alkalescens Mycoplasma bovigenitalium Mycoplasma canadense Mycoplasma californicum Staphylococcus aureus Streptococcus agalactiae 	 Pathogens identified Staphylococcus aureus Staphylococcus spp. Streptococcus agalacti Streptococcus dysgala Streptococcus uberis Escherichia coli Enterococcus spp. Klebsiella oxytoca and K. pneumoniae Serratia marcescens Corynebacterium bovis Trueperella pyogenes 		

	Interpretation	VeriVet Software			
	Displa	ay plots and well details	Select Assays	Select View	
ection of ogens and in four ctions	The second secon	Results 1 2 3 4 5 6 7 8 4 0 </td <td></td> <td>Image: selection selectio</td>		Image: selection selectio	
	Pat	thogen DNA is detected (belo	ow cut off)	ele henocacus spin C convis	
27	Pat	hogen DNA is not detected			
S	Pat	hogen DNA is detected in qu	antity above the assay	y's cut-off value	
ae octiae	Inte	ernal Amplification Control (IA	C) failed and pathoge	en DNA is not detected	
d/or	Export result (Ex	cel format)			
5		amplo Accav	Torgot Ct. To	raot Pocult Custom Coll	



A1	A1 NEGATIVE VetMAX MastiType Multi 1	S.aureus	0.00	NEGATIVE		
			Enterococcus spp.	0.00	NEGATIVE	
			C. bovis	0.00	NEGATIVE	
		Str. dysgalactiae	27.98	POSITIVE	++	
		beta-lactamase	0.00	NEGATIVE		
A2	POSITIVE	VetMAX MastiType Multi 2	IAC	26.93	POSITIVE	
			Mycoplasma spp.	0.00	NEGATIVE	
			E. coli	0.00	NEGATIVE	
			Str. Agalactiae	0.00	NEGATIVE	
			Str. Uberis	0.00	NEGATIVE	
A3	POSITIVE	VetMAX MastiType Multi 3	Prototheca spp.	0.00	NEGATIVE	
		Staph. Spp.	32.82	POSITIVE	+	
			IAC	23.74	POSITIVE	
			T.pyo-Pstr.indolicus	0.00	NEGATIVE	
A4 POSITIVE		Yeast	33.52	POSITIVE	+	
	VetMAX MastiType Multi 4	Serratia marcescens	0.00	NEGATIVE		
		IAC	20.38	POSITIVE		
			Klebsiella spp.	0.00	NEGATIVE	
			M. bovis	0.00	NEGATIVE	
			IAC	28.65	POSITIVE	
A5 POSITIVE	VetMAX MastiType Multi 1	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	
A7 POSITIVE		Str. Uberis	37.64	SUSPECT		
	VetMAX MastiType Multi 3	Prototheca spp.	0.00	NEGATIVE		
		Staph. Spp.	28.51	POSITIVE	++	
		IAC	23.39	POSITIVE		
		T.pyo-Pstr.indolicus	0.00	NEGATIVE		
		Yeast	0.00	NEGATIVE		
A8	NEGATIVE	VetMAX MastiType Multi 4	Serratia marcescens	0.00	NEGATIVE	
			20.01	POSITIVE		
			IAC	20.01		

M. bovis

IAC

0.00 NEGATIVE

29.55 **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

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Characteristics of complete method			The MastiType workflow takes about 3 hours.
Number of target tested	Positive	289	Acid Purification Kit has been validated or
on milk field samples	Negative	1059	validated on the 3 different 4-plexed kits called
Similar results (%) MastiType workflow compared to another workflow	> 95%		 provides equivalent results on the 7500 and Systems. The Thermo Fisher Scientific mastitis solution to handle and can provide rapid and accurate the efficacy of surveillance and control program

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

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