



Simple, fast and complete solutions for mastitis identification

October 20, 2018

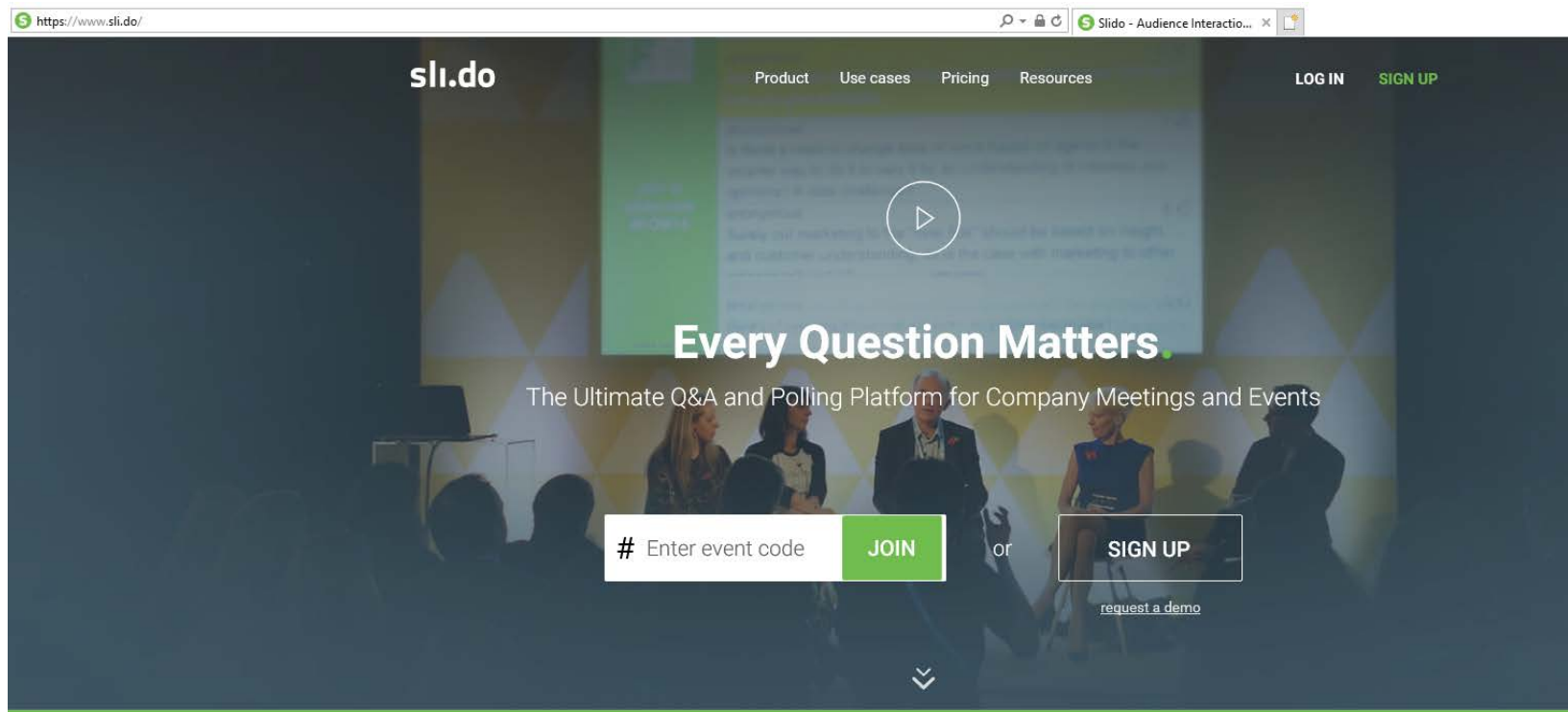
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Simple, fast and complete solutions for mastitis identification

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- Dr. Christina Boss, Product Manager bovine diagnostics:
Challenges of mycoplasma mastitis
- Quoc Hoang - R&D scientist:
Fast and convenient nucleic acid extraction from milk samples
- Rohan A. Shah - R&D Field Support Lead:
Standardize data analysis with cloud-based VeriVet software

Does your group / lab test for mastitis?

- Yes
- No

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#1

- a) Mastitis is the number one costly disease in cattle
- b) Mastitis was the first disease to be discovered in cattle

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#1

US\$19.7-32 billion

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US\$140 million

- a) Total *M. bovis* estimated disease cost annually in the U.S.
- b) The total annual cost for mastitis in the U.S.

#1

US\$19.7-32 billion

US\$ 385-770

US\$140 million

Bacterial culture



Long time to result –
Up to 10 days



No-growth samples –
Up to 40 %



No mastitis pathogen
identified



Results obtained within a day, much shorter than with culture

Bovine Mastitis – Pathogen identification

- **MagMAX CORE Nucleic Acid Purification Kit and Mastitis & Panbacteria Module** for fast and convenient nucleic acid extraction from milk samples, yielding high-quality nucleic acids



Major *Mycoplasma* species infecting cattle

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

Major contagious mastitis-causing pathogens

Mycoplasma bovis
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus uberis

• Major mastitis-causing pathogens

- *Staphylococcus aureus*
- *Staphylococcus* spp. (including all major coagulase-negative staphylococci)
- *Streptococcus agalactiae*
- *Streptococcus dysgalactiae*
- *Streptococcus uberis*
- *Escherichia coli*
- *Enterococcus* spp. (including *E. faecalis* and *E. faecium*)
- *Klebsiella oxytoca* (and/or *K. pneumoniae*)
- *Serratia marcescens*
- *Corynebacterium bovis*
- *Trueperella pyogenes* and/or *Peptoniphilus indolicus*
- *Staphylococcal* β -lactamase gene
- *Mycoplasma bovis*
- *Mycoplasma* spp.
- Yeast
- *Prototheca* spp.

Which test do you use to test for mastitis?

- a) Bacterial culture
- b) PCR
- c) Both PCR and bacterial culture
- d) None of these

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The last sample extraction method you may ever need

- Flexible solution designed to meet lab's current and future testing needs
- For widest range of animal health sample matrices
- Helps make labs more efficient
- Magnetic bead-based sample extraction
- Automatable on KingFisher magnetic particle processors
- CORE chemistry + Modules



For Laboratory use including veterinary and environmental uses.

Together with the Mastitis & Panbacteria Module for milk samples the MagMAX CORE workflow works with:

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

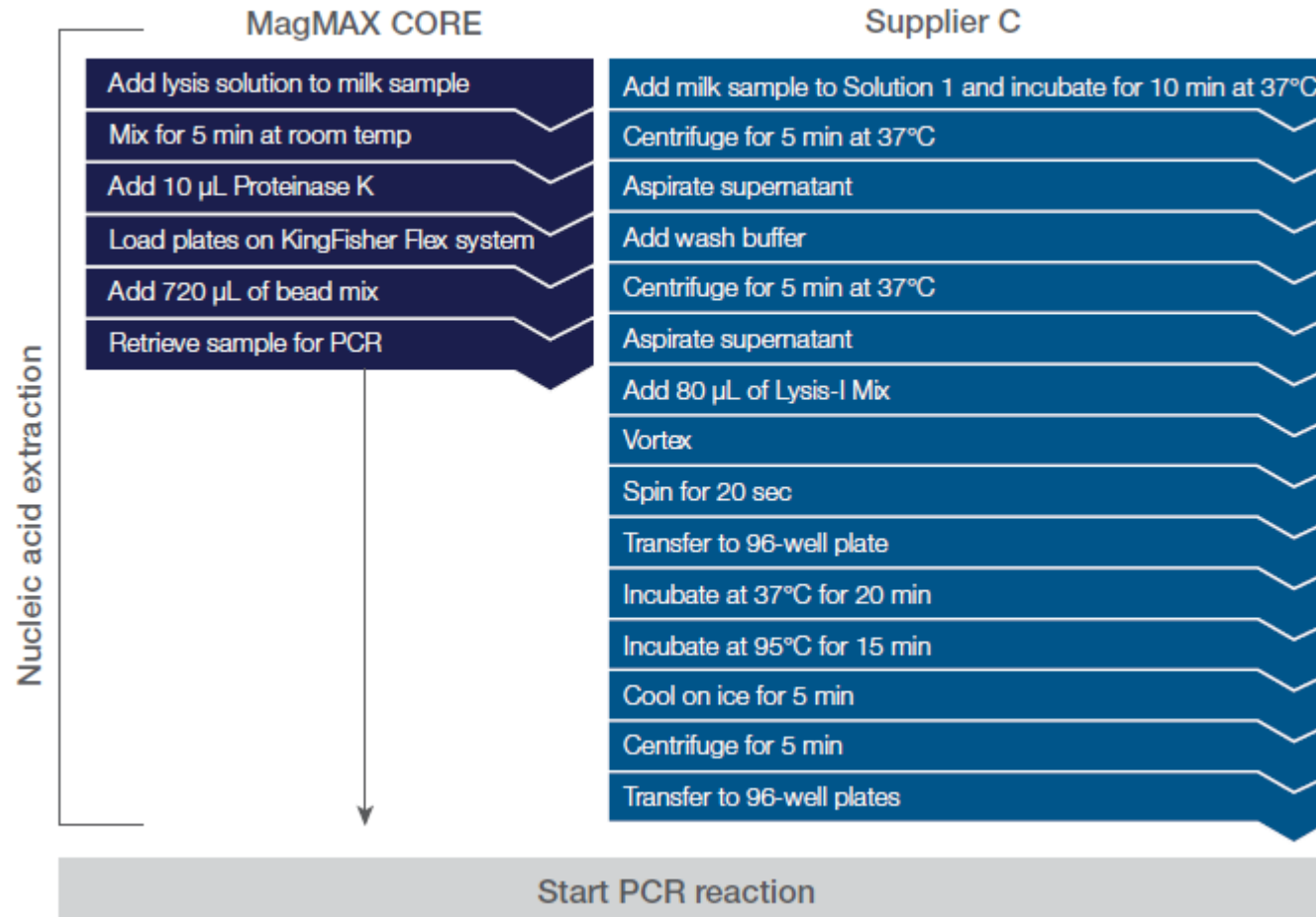


*Use of preserved milk samples eliminates the need for cooled transport of samples

See **Poster # 36** for detailed data (copies of the poster are available)

MagMAX CORE Mastitis & Panbacteria Module

- Fast: 96 samples in 1 hr
- Minimal hands-on time
- No aspiration
- No centrifugation



- 96 samples in >2 hrs
- Many hands-on steps
- 2x supernatant aspiration steps
- 3x centrifugation steps

What is the benefit of a specialized nucleic acid extraction for mastitis samples?

- a) No benefit
- b) It works with thick/cluttered milk
- c) Multiplexing PCR reactions require high quality nucleic acid
- d) I don't know

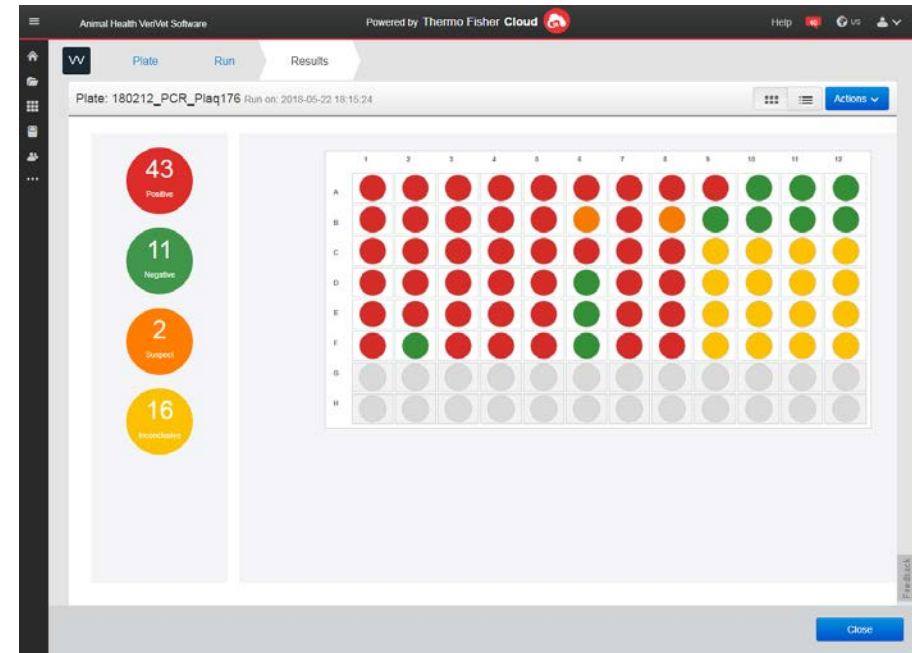
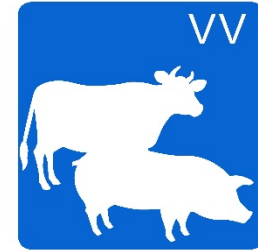
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Convenient VeriVet result interpretation software

Applied Biosystems VeriVet Software

Simplify, accelerate, and standardize data analysis.

- **Ease of use** Innovative cloud-based data analysis and storage
- **Easy read-out** of positive and negative results
- **Quality control:** Inhibited samples identified



For research use only. Not for use in diagnostic procedures.

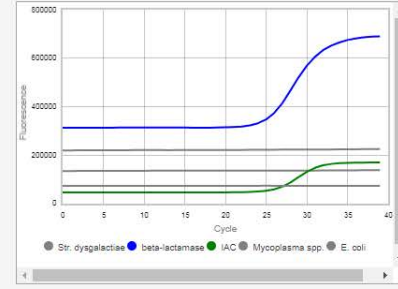
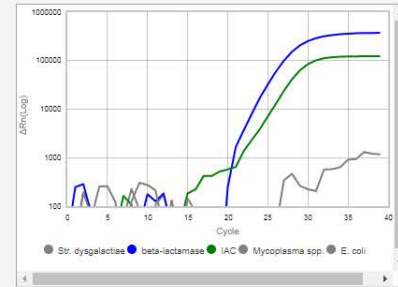
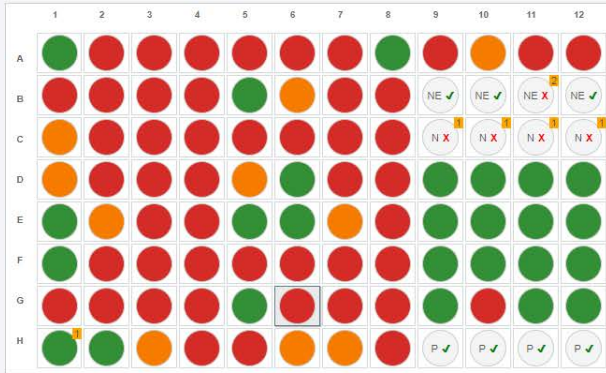


Color results depending on call status

48
Positive

26
Negative

10
Suspect



48

Positive

➤ At least 1 target gives a positive result (below cut off)

26

Negative

➤ Target for pathogens tested are negative

10

Suspect

➤ At least 1 target give a negative call but there is a Ct above cut off

38

Inconclusive

➤ Result not valid, needs retesting

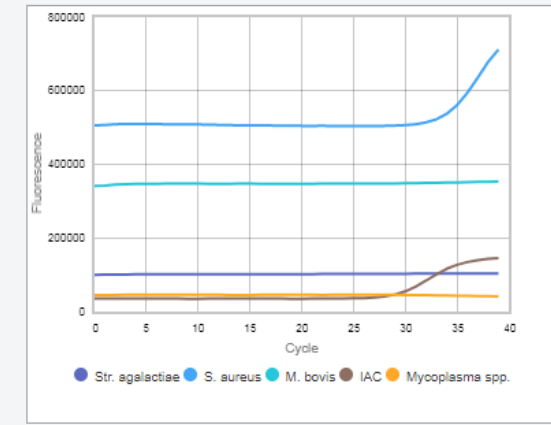
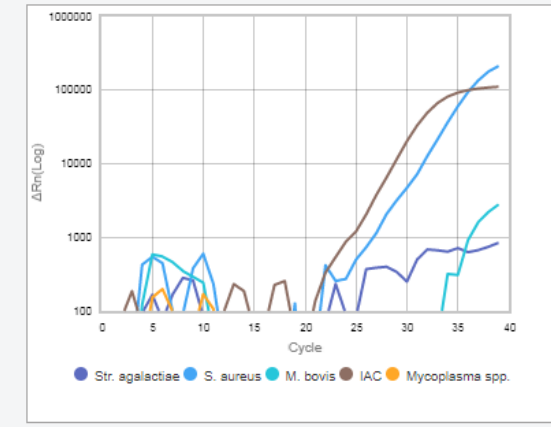
Each dot corresponds to one well

See **Poster # 31** for detailed data (copies of the poster are available)

VeriVet result table

- Unknown Wells									
Well	Result	Sample	Assay	Target	Ct	Target Result	Custom Call	Task	Flag
A1	POSITIVE	A (high)	PCR	Str. agalactiae	0.00	NEGATIVE	+	UNKNOWN	
				S. aureus	35.39	POSITIVE		UNKNOWN	
				M. bovis	0.00	NEGATIVE		UNKNOWN	
				IAC	29.41	POSITIVE		IPC	
				Mycoplasma spp.	0.00	NEGATIVE		UNKNOWN	
A2	POSITIVE	E (high)	PCR	Str. agalactiae	0.00	NEGATIVE		UNKNOWN	
				S. aureus	0.00	NEGATIVE		UNKNOWN	
				M. bovis	0.00	NEGATIVE		UNKNOWN	
				IAC	26.00	POSITIVE		IPC	
				Mycoplasma spp.	22.25	POSITIVE		UNKNOWN	

+ Control Wells



Can the interpretation software give multiple positive calls in one sample?

a) Yes

b) No

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Which of the following answers apply to mastitis?

- a) All types of mastitis are the same
- b) Some mastitis pathogens are more difficult to identify than others
- c) All pathogens require the same action from the veterinarian
- d) Mycoplasma does not require specific culture media

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Which parts of the workflow are needed for identification of mastitis pathogens?

- a) Sample extraction
- b) DNA amplification
- c) Result interpretation
- d) All of the above

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What type of samples can be tested with PCR to identify mastitis pathogens?

- a) Only fresh milk samples
- b) Frozen, preserved and fresh milk samples
- c) Preserved milk samples
- d) Preserved and fresh milk samples

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Q&A