Validation of the PathoProof™ Mastitis PCR Assay for Bacterial Identification from Milk Recording Samples

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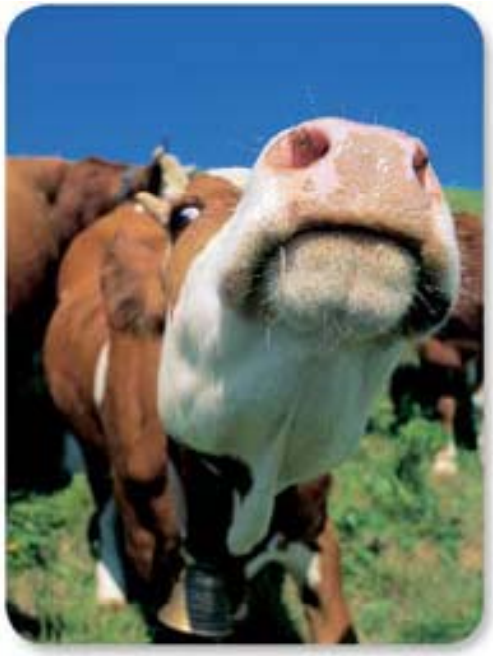
Finnzymes Oy



- Benefits of using DHI samples for mastitis testing
- Overview of the PathoProof Mastitis PCR Assay
- Main questions in using PCR with DHI samples
- Validation with DHI metered milk samples- focus on *Staph. aureus*

Finnzymes Oy

- Founded in 1986
- Headquarters in Espoo, Finland
- Among the world's leading molecular biology reagent manufacturers
- Focus on PCR (Polymerase Chain Reaction) based products since 1989

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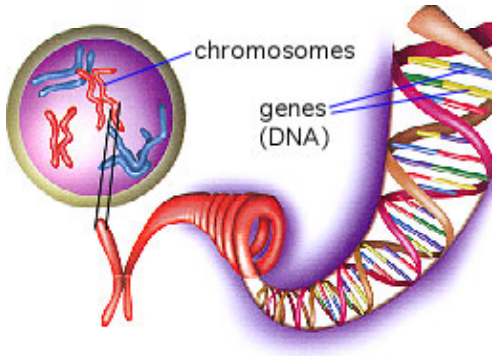
Real-time PCR will change mastitis testing

- Elevated SCC is an indication of mastitis, which is the most important infectious disease in dairy cattle
- Identification of mastitis bacteria is the cornerstone for targeting antimicrobial therapy and an important tool for herd management. Mastitis testing is done in large numbers using bacterial culturing
- DHI samples have not been traditionally used for mastitis testing
  - preserved milk cannot be used for bacterial culturing
- Real-time PCR targets bacterial DNA
  - -live or dead bacteria
  - -preserved milk samples
  - -DHI samples can be used



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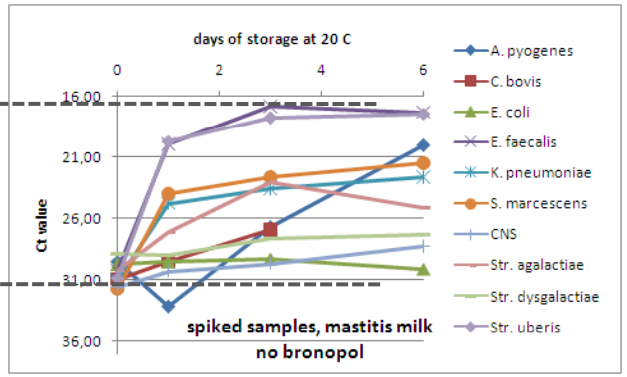
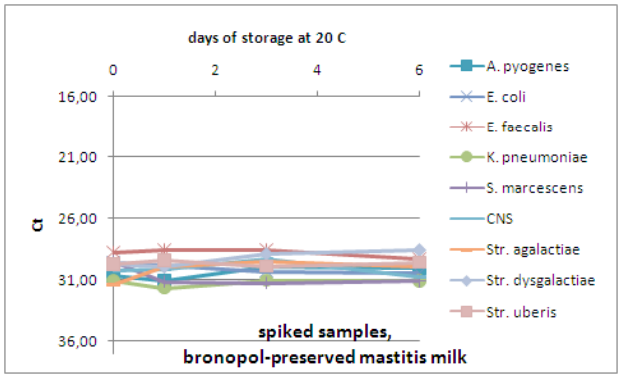
Benefits of using DHI samples for mastitis testing

- Fast detection of mastitis bacteria (currently up-to 7 days with bacterial culturing)
  - -early detection of problems and risks for herd management
  - -improved treatment efficiency and less unnecessary use of antimicrobials
  - -potential for huge cost savings for the producer (currently mastitis costs ~150-200 USD per cow per year)
- Very convenient for the producers. Repeated and laborious sampling is eliminated
- Bacterial growth during sample transportation is no longer an issue

1000x increase in bacterial quantity





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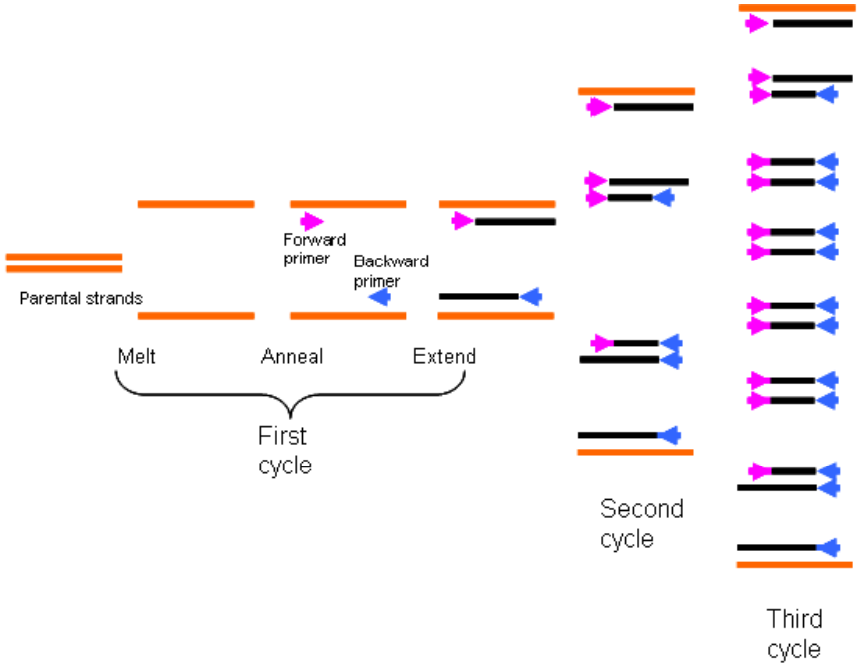
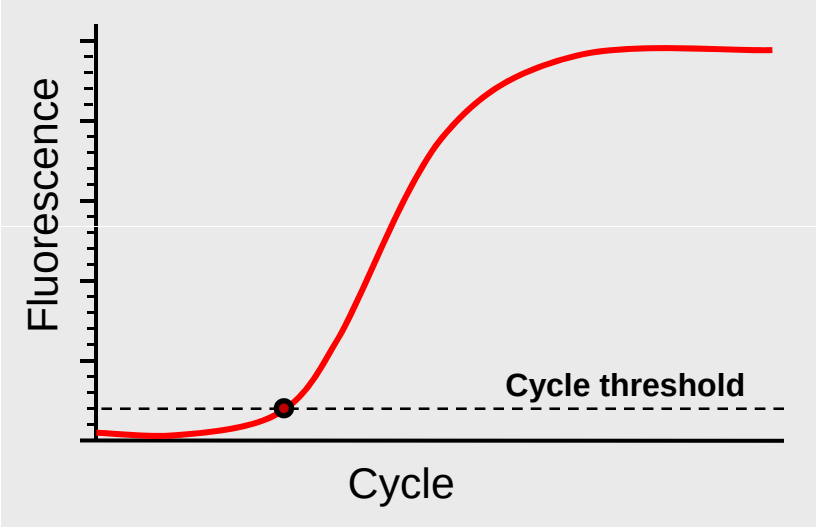
Real-time PCR (Polymerase Chain Reaction)





Real-time PCR is the golden standard of clinical diagnostics

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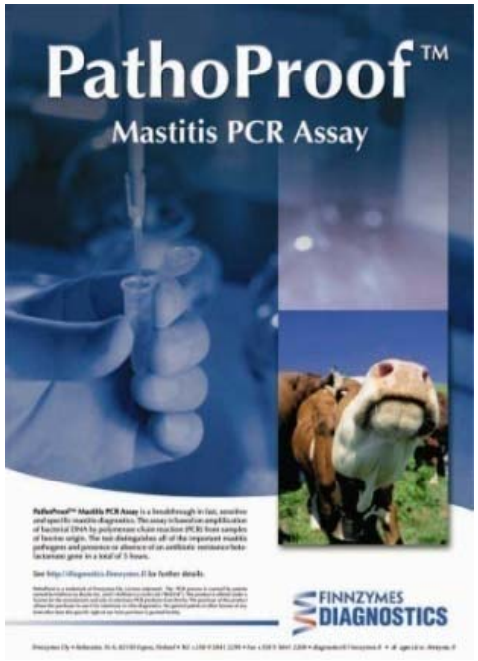
PathoProof Mastitis PCR Assay identifies all important mastitis bacteria in 4 hours

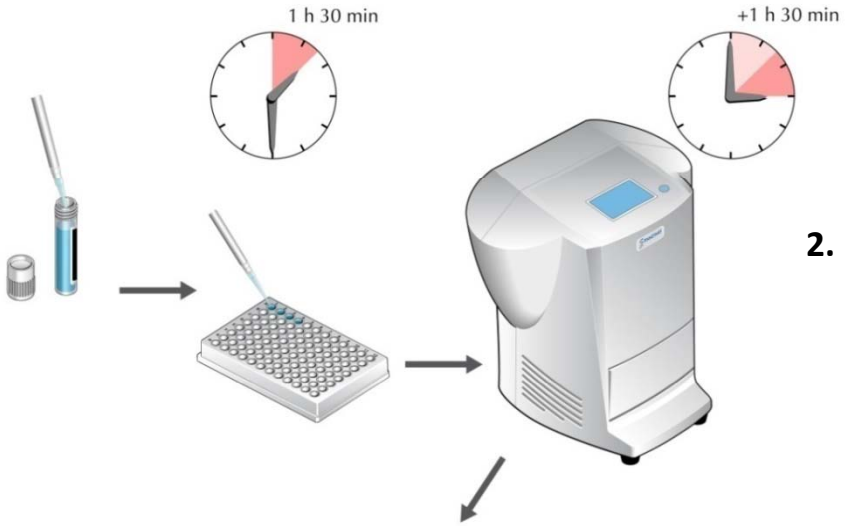
-*Staphylococcus aureus*
-Coagulase negative staphylococci (CNS)
-*Streptococcus agalactiae*
-*Streptococcus dysgalactiae*
-*Streptococcus uberis*
-*Escherichia coli*
-*Corynebacterium bovis*
-*Enterococcus faecalis, E. faecium*
-*Klebsiella pneumoniae, K. oxytoca*
-*Serratia marcescens*
-*Arcanobacterium pyogenes, Peptostreptococcus indolicus*
-Beta-lactamase penicillin resistance gene (blaZ)



⇒ Large-scale data demonstrate that the target bacteria cover >99% of all subclinical and clinical mastitis cases in Europe, as well as in North America (eg. Makovec & Ruegg 2003; Pitkälä et al. 2004; Tenhagen et al. 2006; Koivula et al. 2007)

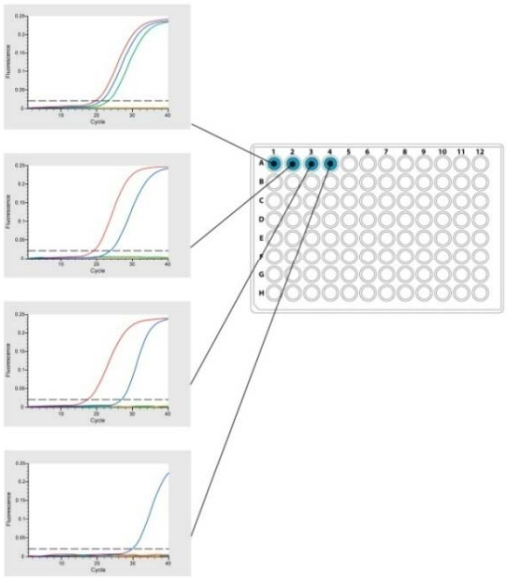
PathoProof Mastitis PCR Assay laboratory workflow

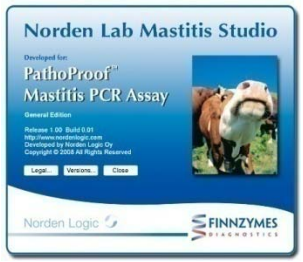
1. **Extraction of bacterial DNA from fresh or preserved milk.**



2. **Real-time PCR amplification of bacterial DNA**

3. **The PathoProof Mastitis PCR Assay identifies and quantifies simultaneously 11 most important mastitis species/groups and the most relevant antibiotic resistance gene (blaZ)**





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Questions in using PCR with DHI samples

1) What is the level of concordance in bacterial detection from milk recording samples using the PathoProof Mastitis PCR Assay, compared to bacterial culture-based testing from udder samples?

(=how do the results compare with the results of the conventional mastitis test)

2) Is there a risk that carry-over milk from one sample to the next can result in false positive bacterial results in DHI sampling?

Validation with DHI metered milk samples- focus on *Staphylococcus aureus*

- ***Staph. aureus*:**

-a severe contagious mastitis pathogen. One of the most important causes of chronic, clinical and subclinical mastitis worldwide

-present in most herds and presents a big problem for mastitis management

-usually low prevalence (few cows in a herd are positive)

-cyclic shedding, repeated sampling is often required to avoid false negatives with bacterial culture

-routine identification of positive cows would be very beneficial for mastitis management

Photo: Danish Dairy Board

Staph. aureus identification from DHI samples

(Kelton et al., in preparation)

- Five Ontario dairy herds with *Staph. aureus*
- Sampled 241 cows on DHI test day
- Three samples from each cow:

1) Aseptically collected udder composite sample- bacterial culture
2) Aseptically collected udder composite sample- PathoProof Mastitis PCR Assay
3) Metered (TrueTest) DHI sample with preservative- PathoProof Mastitis PCR Assay

- Milking order and unit number were recorded

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Staph. aureus identification from DHI samples

- 35/241 udder samples were positive for *Staph. aureus* in culture. Of these, 34 were positive with the PCR assay
- Of the 35 culture positive animals, 32 metered DHI samples were positive for *Staph. aureus*. Excellent agreement!
- Additional 9 culture negative samples were positive in DHI sample PCR. Possible considerations:

  1) PathoProof Mastitis PCR Assay from DHI samples had higher sensitivity in *Staph. aureus* identification than bacterial culture from udder samples (cyclic shedding of *Staph. aureus* is known to produce false-negative culture results)?

  2) carry-over from previous positive sample resulted in false positive PCR?

  ~~3) false positive signals in real-time PCR?~~ (PathoProof PCR Assay has 100% analytical specificity. *Journal of Dairy Science* 92: 952-959)

Photo: Danish Dairy Board

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Staph. aureus identification from DHI samples

Composite culture *Staph. aureus* positive samples: 32
DHI PCR *Staph. aureus* positive samples: 41

		Composite culture	
		Positive	Negative
DHI PCR result	Positive	32	9
	Negative	3	197

Sensitivity = 32 / 35 = 91.4 %
Specificity = 197 / 206 = 95.6%

But: follow-up investigation of the 9 culture negative, DHI PCR positive samples!

Follow-up of culture negative samples that were positive in DHI PCR

- Follow-up investigations to determine if these cows were really *Staph. aureus* positive, but cultured negative on this occasion
- 6/9 culture composite negative but DHI PCR positive animals were available for re-sampling and re-culture
- 3/9 samples were not available (high SCC cows with mastitis: culled or dried off and dry-treated before we could re-sample)

Cow ID	SCC	DHI PCR	PCR-Comp	Culture Comp	Repeat Culture
Bryn	700	+	-	-	NA
Carla	2311	+	-	-	NA
711	3311	+	-	-	NA

Follow-up of culture negative samples that were positive in DHI PCR

- Out of the 6 available re-samples, 3 now provided culture-positive results!

Cow ID	SCC	DHI PCR	PCR-Comp	Culture Comp	Repeat culture
88	104	+	-	-	+
305	136	+	-	-	+
720	901	+	+	-	+

- Earlier ID of *Staph. aureus* infected cows using DHI PCR!!

Carry-over analysis

- Out of the 6 DHI PCR positive but culture negative cows, 3 cows were milked with the same unit after herd mates who were DHI PCR positive (for the remaining 3 cows, carry-over was not possible as previous herd mates were negative for *Staph. aureus*)
- Comparison of bacterial quantities in the samples indicated that carry-over could be excluded for 2/3 of the 'suspect' samples
- Carla was the only animal (out of 241!) for which carry-over could not be excluded. (note: Carla had mastitis and SCC of >2 million)

Cow ID	SCC	DHI PCR	PCR-Comp	Culture Comp	Repeat Culture
Olive	182	+++	-	-	-
Carla	2311	+	-	-	NA
711	3311	+	-	-	NA

To conclude...

- Real-time PCR –based *Staph. aureus* identification from metered DHI samples is at least as sensitive and accurate as composite milk culture
- In addition, some true positive cows were identified by DHI PCR, but not by composite culture
- Carry-over was not an issue for DHI PCR of *Staph. aureus*
- PathoProof Mastitis PCR Assay from DHI samples is a highly convenient and reliable tool for mastitis management. Results are easily integrated with SCC history

Photo: Danish Dairy Board

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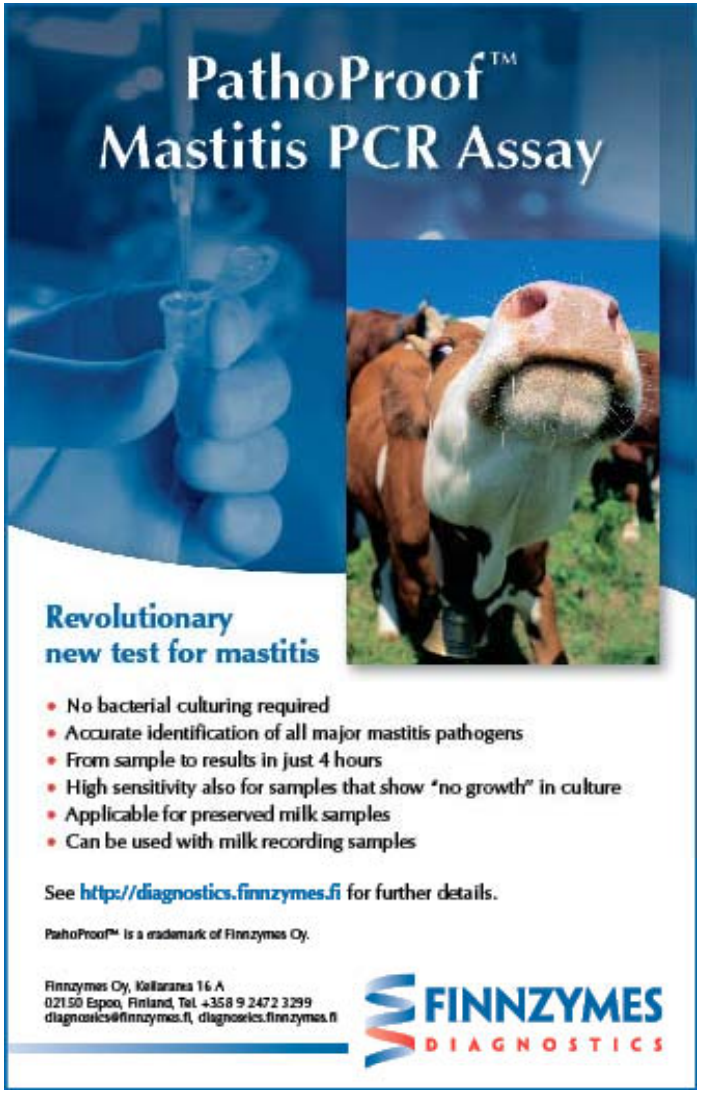
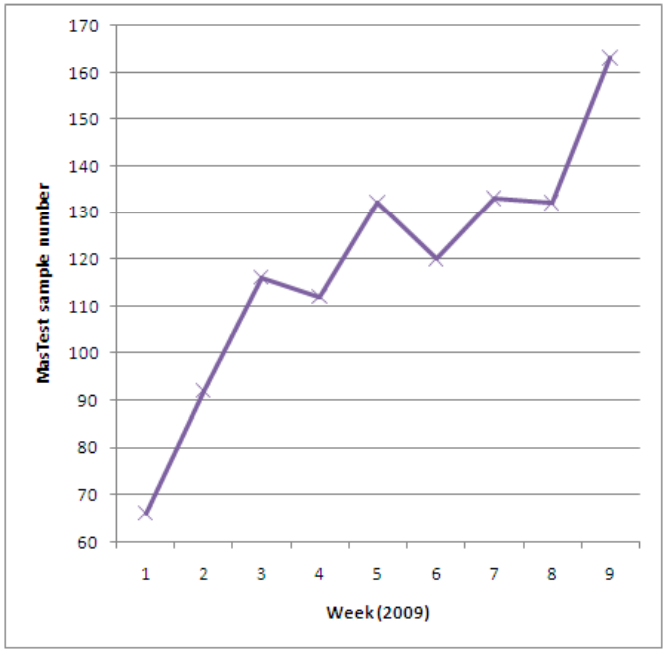


- Testing running and available now in 9 countries
- Strong demand from dairy producers
- Convenience and speed are among the key benefits recognized by the producers



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- Danish Dairy Board: Jorgen Katholm
- Eurofins Steins: Soren Jensen, Carsten Gronbaek

Dairy

CanWest DHI now offering a convenient test for mastitis

The DNA test can be done using the same milk sample currently used to test for somatic cell counts

BY FRANCES ANDERSON
Ontario Farmer

Mastitis testing will become as easy as taking a milk sample, thanks to a new service being offered by CanWest DHI.

"We're going to be the first DHI in the world to launch such a service," Richard Cantin told Ontario Farmer last week.

The PCR-DNA technology is used in Finland and Holland, but it's used by traditional animal health laboratories, as opposed to milk recording companies.

"Convenience" is the big advantage of using a DNA test for Staphylococcus aureus (Staph. A.) The test will be done using the same milk sample that DHI collects for SCC (somatic cell count) and composition testing.

Cantin said he expects milk producers will choose one of three approaches to testing: testing the whole herd, testing selected cows, such as newly purchased animals, or cows with clinical signs of mastitis, or testing samples that exceed a specified SCC threshold.

"We worked with five herds, making up close to 300 cows to validate the test," said Cantin, who is DHI's manager of marketing and customer services. They compared the results of the DNA testing with the results of culturing samples and found that the DNA testing is more accurate, since there are some false negatives in cultured samples where there's no bacterial growth.

The other advantage of DNA testing is its speed. Because you don't have to wait for bacteria to grow, the samples can be tested within three or four hours of arriving in the lab, and producers will get a report the next day.

Because the testing is done by DHI, the report will include SCC, and production history, like days in milk, to "enhance" the results, said Cantin.

While DHI always recommends consulting with the veterinarian regarding herd health management, most producers are fairly well aware of how to manage Staph A. when it's identified, Cantin said. This is a contagious form of mastitis and is most commonly spread through the milking routine.

"There's pretty high value in managing the cows to prevent this," milking infected cows last, for example.

The speed, convenience, and accuracy, come at a cost. DHI had not yet determined the price, but Cantin said "it's probably going to be in the $25 to $30 (a sample) range, which is typical for DNA tests."

This is a completely different test than DHI offers for Johnes and Leucosis, which are based on ELISA technology.

The DNA testing for mastitis requires the purchase of specialized equipment and changes to the laboratory to accommodate it, in mid to late December.

"Lab capacity is an issue with this type of test, so it will be a staged roll-out," said Cantin. The service will most likely be introduced region by region, as the lab can test only 200 milk samples for mastitis a day, out of the 8,000 to 10,000 milk samples it receives daily.

"We continue to look at other diseases that can be monitored through the DHI samples," said Cantin.

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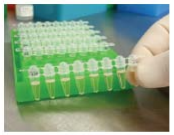
Improvements in udder health leads to...

- *INCREASED MILK PRODUCTION*
- *REDUCED TREATMENT COSTS*
- *REDUCED MILK DISCARD*
- *REDUCED PREMATURE CULLING*
- *LOWER SOMATIC CELL COUNTS*
- *IMPROVED MILK QUALITY*



Early detection of mastitis infections is important, especially when dealing with a contagious pathogen such as Staph. aureus. The ability to fully integrate SCC and Staph ID test information can enhance decision making at the farm and improve udder health, milk quality and your profitability.

Producers should work closely with their veterinarian to design mastitis best management practices, determine a testing plan for their herd, test results interpretation and implementation of an action plan for test positive cows.

Staph iD
STAPH AUREUS IDENTIFICATION

For more information on the Staph ID testing service, please talk to your DHI field staff or contact DHI at 1-800-549-4373.

For more information on mastitis control talk to your veterinarian or visit the National Mastitis Council at www.nmconline.org.

CANWEST DHI

660 Speedvale Avenue West
Guelph, Ontario
1.800.549.4373
www.canwestdhi.com

Testing your cows for mastitis just got a whole lot easier.

Staph iD
STAPH AUREUS IDENTIFICATION

New mastitis test from CanWest DHI

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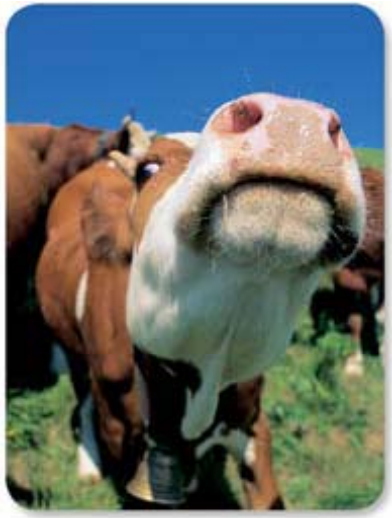
Thank you for your attention!



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