

New Testing Technologies: Our Journey into DNA Testing



NALMA Meeting
October 12th - 2009

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Agenda

1. Introducing New Technologies
2. The New DNA test
 - Validation Work
 - Setting Up the Lab
 - Launching the Test
3. What's next?



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Introducing New Technologies

Our Philosophy and Approach

- Get as much useful information out of samples coming to the lab as possible
- New technology must be:

Validated – against traditional methods and on our COWS

Approved by Veterinarians – considered to be our customers as well

(When it comes down to Disease management, Herd Veterinarians play a key role in test results' interpretation and 'Best Management Practices' recommendations...)

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Disease Testing Focus

Johnes

- Started accepting samples in Spring 2005

Leukosis

- Started accepting samples in Spring 2007

DNA Analysis

- Started accepting samples for *S. aureus* identification March 1st 2009 (Staph-ID)



Why DNA analysis? Why RT-PCR? (Real Time Polymerase Chain Reaction)

- DNA can be detected even if bacteria is dead, hence being able to use the preserved DHI sample
- PCR tests detect and amplify particular sequences of DNA specific to target in question – quick TAT for results (2.5 hours extraction, 1.5 hour machine time)
- Commercial mastitis test developed by international diagnostics companies (Finnzymes)



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Why DNA analysis? Why RT-PCR? (Real Time Polymerase Chain Reaction)

- RT-PCR technology validated in published studies
- It is a closed system, thus less susceptible to cross-contamination – more robust test
- Validated on preserved metered samples i.e. routine DHI samples



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Timeline

- Fall 2007 – send mastitis culture samples from Ontario cows to Finnzymes
- January 2008 – demonstration of PathoProof assay at Gallant Labs
- Summer/Fall 2008 – validation study



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Timeline

- Fall 2008 – design, build, equip lab
- Winter 2009 – hire lab technologist, Finnzymes training, start accepting samples (March 1st)
- Fall 2009 – new testing technology audited by Standards Council of Canada
- Early 2010 – accredited status achieved for PCR test

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Validation Study Summary

- 2 vet clinics, 5 herds, 229 cows
- On test day... collected samples for milk cultures (aseptic, prior to milking), collected regular DHI sample & kept track of milking order/meter order
- 3 results for each cow... teat end culture, teat end PCR, DHI PCR



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Validation Study Summary

Results...

- 34 cows cultured +ve for *S. aureus* (15%)
- ALL 34 cows PCR +ve with teat end sample
- 32 +ve with DHI PCR (other 2 borderline)



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Validation Study Summary

Results...

- 13 additional cows were PCR +ve (culture neg) with teat end and/or DHI sample...
- 9 of those cows were available for quarter re-cultures... 5 cultured +ve in at least 1 quarter
- 4 unavailable cows... 1 dry, 1 logistics issue, 2 sold for mastitis



Validation Study Summary

Results... Possible carry over of milk (claw, hose, meter, lab) from cow to cow and possible false +ve results?

- 4 cows with DHI +ve and culture/PCR –ve that followed +ve cows on all tests (i.e. carry over?)
- 3 of 4... carry over was ruled out by either dilution calculations (would have needed $>10\%$ milk carryover to achieve that change in Ct value) or by follow up culture.
- 1 of 4... could not rule out, but... cow had SCC of 2.3M and could not be recultured (dry)



Validation Study Summary

Conclusions...

- PCR is at least as sensitive/accurate as cultures and likely more
- Routine metered, preserved DHI samples can be used effectively with the PCR technology
- Although not ruled out 100%, possible false +ve due to milk carry over is unlikely, or at least at low probability



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Testing Options

1. Whole herd
2. Specific cows (new cows, clinical cows...etc)
3. High SCC cows that will be redirected from SCC analyzer for Staph ID test.

Consult with your Vet



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PLEASE INCLUDE THIS FORM WITH SAMPLES

Herd Name: _____ DHI Herd Number: _____

Province: _____ DHI Test Date: _____

DHI Staff Name: _____ Employee Number: _____

PLEASE PERFORM STAPH ID TEST ON THE FOLLOWING:

1 Whole Herd/All Samples (DHI Staff - highlight ALL vials with PINK marker)

Cows as listed on this form (DHI Staff - highlight APPROPRIATE vials with PINK marker)

2 *AND/OR*

Cows on this test with SCC greater than 200k 500k 750k 1.0m or SCC greater than

(Lab Staff - Please set aside appropriate vials based on SCC value)

RETURN RESULTS VIA
(Check ONLY ONE)

Mail email Fax

VETERINARIAN DETAILS

(DHI will automatically send test results to your veterinarian)

Producer email Address

Veterinarian Name

Producer Fax number

Clinic Name

LIST OF COWS FOR STAPH ID TEST

Chain or Cow Name	Vial #
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Chain or Cow Name	Vial #
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Continue to list cows on back side if needed. Please CLEARLY indicate the cow ID and barcode #



Main Testing area – 3 testing lines









Extracted DNA – ready for replication









Overview – The Service

- Uses SAME DHI milk sample
- Samples to Guelph lab as per normal process (Ont direct, West after tested for F/P/SCC/MUN locally)
- Results available within 2-4 days of sampling
- Individual cow results report
- Results MUST also be provided to your Veterinarian
- Results remain confidential (same as all DHI info!)



Staph ID Test

Cost...

- \$29/sample – No herd/handling fee
- Efficiency volume discount:
 - 25-49 samples: -\$1
 - 50-74 samples: -\$2
 - 75-99 samples: -\$3
 - 100+ samples: -\$4



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Staph ID Test

Cost... Compared to traditional culture...

- Not that far off what some herds pay for cultures...
- No sample collection costs
- Reliability of results...?
 - Less resampling of ‘no growth’ cultures (25-40% of time)
- Ability to integrate with SCC information
- Results in DHI database
- **CONVENIENCE!**

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Staph ID Test

To date...

- 4500 samples tested since March 1, 2009
- Repeat customers
- Customers that want to send in samples between regular DHI tests



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What's Next?

Other 'contagious' pathogens

- Short term... Strep Ag. & Mycoplasma (+ beta lactamase gene)
- Longer term... Bulk Tank Screening

All mastitis pathogens



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What's Next?

Additional Diseases

- Currently: Johnes & Leukosis
 - BVD
 - Neospora
 - Others...?
-
- Bulk Tank Screening
 - PCR Technology opens that door...



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Concluding Remarks

Setting up new testing technology in the lab can be:

- A. a lengthy process
- B. challenging
- C. fun and exciting!
- D. all of the above





**Thank
You!**

Questions?

