Real-Time qPCR-based DNA Mastitis Analysis using the preserved DHIA sample

Jere High

CEO

Lancaster Dairy Herd Improvement Association

WWW.LANCASTERDHIA.COM

Jere@LancasterDHIA.com

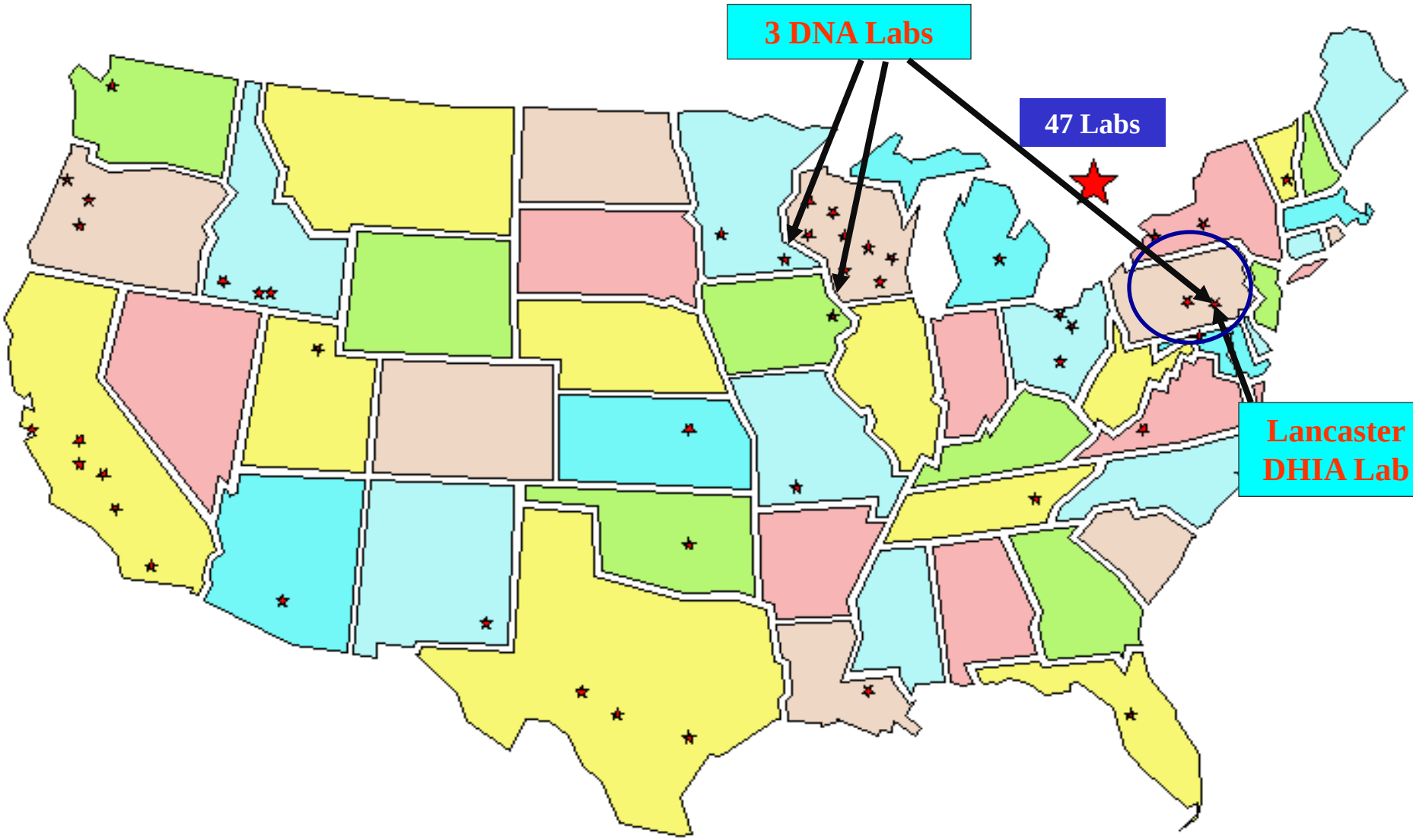
President

North American Lab Managers Association

WWW.NALMA.ORG

ICAR 37th Annual Meeting – Riga, Latvia 2010

DHIA Laboratory Locations



Lancaster DHIA

Core Purpose

To help our members, and the agriculture community, prosper while promoting a safe and abundant food supply.

Core Values

Teamwork, Integrity, Golden Rule, Pride and Innovation

15 Dairy Producer Member Board (2,900+ Members)

10 Employee Management Team

50 (f/p) Field Technicians (209,000 cows)

7 DHIA Lab Techs (167,000 samples)
6 Microbiology Techs (1,200 PLC)
5 Office
8 (p/t) van drivers (3 routes)

Field Services

DHIA Testing

Management Consulting

Lab Services

DHIA Lab

Microbiology Lab

Culture Lab

DNA Mastitis Lab

Lancaster DHIA Office

Using DHIA records to help you make management decisions

- How does cutting my SCC improve my milk production?
- With a SCC of 400,000 or higher
- Cut your SCC in half can increase your production by an average 1.5 pounds or .68 kilograms per day per cow

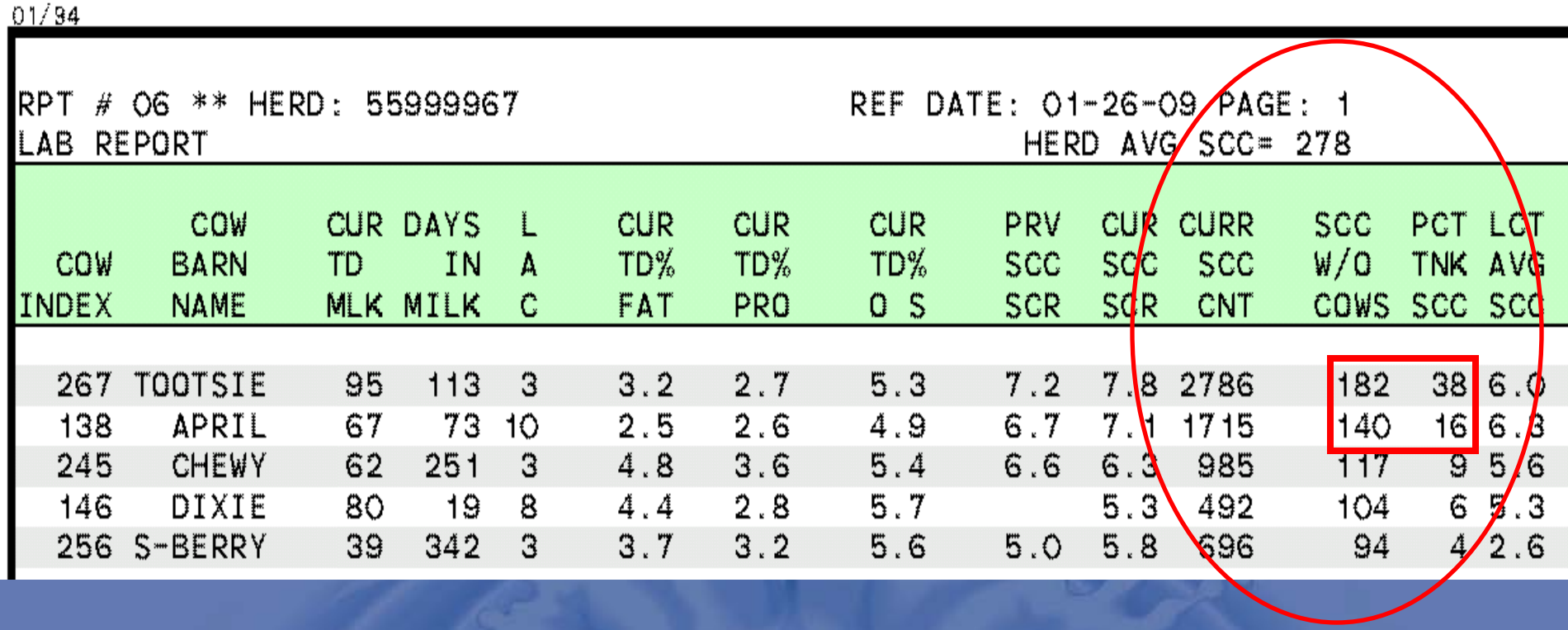
- **Milking 50 cows will add 75 pounds per day or 34 kilograms per day**
- **75 lbs per day @ $16 cwt = $12 / day or €13.12 / 45 kilograms = €9.84 / day**
  - **(Ls9.12 / 45 kilograms = Ls6.96 / day**
- **Per year = $4,380 / €3,592 / Ls2,540**
- **Add a $0.40 bonus = $5,110 / year**

  €0.33 / kilogram = €4,190 / year

  Ls0.23 / kilogram = Ls2,964 / year
  - **(70 lbs or 32 kgs /cow/day average)**

A total of
$9,490 / €7,782
Ls5,504
by just lowering your SCC
by half

Where do I look first?



01/34

RPT # 06 ** HERD: 55999967 REF DATE: 01-26-09 PAGE: 1
LAB REPORT HERD AVG SCC= 278

COW INDEX	COW BARN NAME	CUR TD MLK	DAYS IN MILK	L A C	CUR TD% FAT	CUR TD% PRO	CUR TD% O S	PRV SCC SCR	CUR SCC SCR	CURR SCC CNT	SCC W/O COWS	PCT TNK SCC	LCT AVG SCC
267	TOOTSIE	95	113	3	3.2	2.7	5.3	7.2	7.8	2786	182	38	6.0
138	APRIL	67	73	10	2.5	2.6	4.9	6.7	7.1	1715	140	16	6.3
245	CHEWY	62	251	3	4.8	3.6	5.4	6.6	6.3	985	117	9	5.6
146	DIXIE	80	19	8	4.4	2.8	5.7		5.3	492	104	6	5.3
256	S-BERRY	39	342	3	3.7	3.2	5.6	5.0	5.8	696	94	4	2.6

Most times your top 2-5 cows make up 50% of your bulk tank


WANTED ~ DEAD OR ALIVE ~
MASTITIS DNA

What is PCR-based Mastitis Testing?

PCR is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of a particular DNA sequence.

Why use PCR-based Mastitis testing over the standard culture?

In approximately 25–40 % of bovine milk samples taken from animals with clinical mastitis, no bacterial growth can be detected in conventional culturing.

What are the Advantages

- Samples can be sterile but do not need to be
- Using the preserved samples from DHIA testing
- You don’t need to collect a separate sample
- You can pool samples from groups of cows
- Chronic Mastitis cows can be tested

PCR-based mastitis testing leads to more effective treatments by:

- More specifically identifying coliforms
- Identifying sources of mastitis in a single cow or bulk tank sample
- Identifying sources of mastitis in cows already being treated
- Decreasing test time from 2-10 days (conventional culturing) to 1-2 days from when we receive the sample at the lab.

What tests are available?

Full Panel (Complete-12 kit)

12 tests in this analysis

Contagious Pathogens

- ***1. Staphylococcus aureus***
- ***2. Streptococcus agalactiae***

Environmental Pathogens

- ***3. Corynebacterium bovis(Contagious in some cases)***
- ***4. Streptococcus dysgalactiae (Contagious also)***
- ***5. Staphylococcus sp.***

  (including all major coagulase-negative staphylococci)
- ***6. Streptococcus uberis***

Full Panel Cont'd

Environmental Pathogens

- *7. Escherichia coli*
- *8. Enterococcus sp.*
  (including E. faecalis and E. faecium)
- *9. Klebsiella sp.*
  (including K. oxytoca and K. pneumoniae)
- *10. Serratia marcescens*
- *11. Arcanobacter pyogenes and Peptoniphilus*
  (Peptostreptococcus) indolicus

Miscellaneous – Linked to staph species

- *12. Staphylococcal β-lactamase gene*

Contagious Analysis

- *1. Staphylococcus aureus*
- *2. Streptococcus agalactiae*
- *3. Mycoplasma bovis*

Individual or multiple cow selections

- Choose your top SCC cows plus selecting any other cows you are suspicious for mastitis infection. Next the DNA lab will perform the PCR-based DNA test for you.
- Choose cows over your specified SCC level. For instance you can have the DHIA Field Technician mark the DNA Lab Form for anything over 400,000 SCC. You can exclude cows that you do not wish to be tested.
- Testing all fresh cows to prevent chronic mastitis problems.
- Testing treated cows is available since PCR-based DNA will pick up dead or alive Mastitis DNA and help you determine if you need additional extended therapy to clear up a chronic infection.

How Does It Work?

1. Collect milk sample

2. Fill out DNA Lab Form

Lancaster DHIA PCR DNA Mastitis Diagnostics

1592 Old Line Road, Manheim, PA 17545 ~ Office Phone 1-888-202-3442 ~ Fax 717-664-2911
~ DNA Lab 1-877-572-4115 ~ Email DNA@LancasterDHIA.com

DNA Lab Form LDHIA Tech # & Name:_____

LDHIA Herd Code ___-___-_____ Date:_____ Not a LDHIA Customer:_____

Name:_____

Address: _____

_____

_____

Bulk Tank Sample(s) Only List Here

_____, _____, _____, _____, _____

Send Results: Same as billing address ___ or use address below ___

Email:_____

Phone:_____ **Fax:**_____

___ **Send to my Veterinarian (Name)**_____

Phone:_____ **Fax:**_____

Email:_____

Address:_____

Lancaster DHIA Technicians ~ Please circle specific cows requested with RED magic marker on **Lab List**

Please check the box below that applies

See other side for descriptions of Full Panel & Contagious

Full Panel Test Member Price	Contagious Test Member Price	Full Panel Test Non- Member Price	Contagious Test Non-Member Price	Discount applies for more than 30 samples
$24	$19	$26	$21	Please call for pricing

Please check the box below that applies

Whole Herd Test	Cows over _____SCC	Cows over 400k SCC	Top 5 Cows for SCC	Cows listed on back

Additional testing instructions:

3. Samples analyzed for components and SCC in DHIA Lab

4. DHIA Lab “Hot Sheet is used

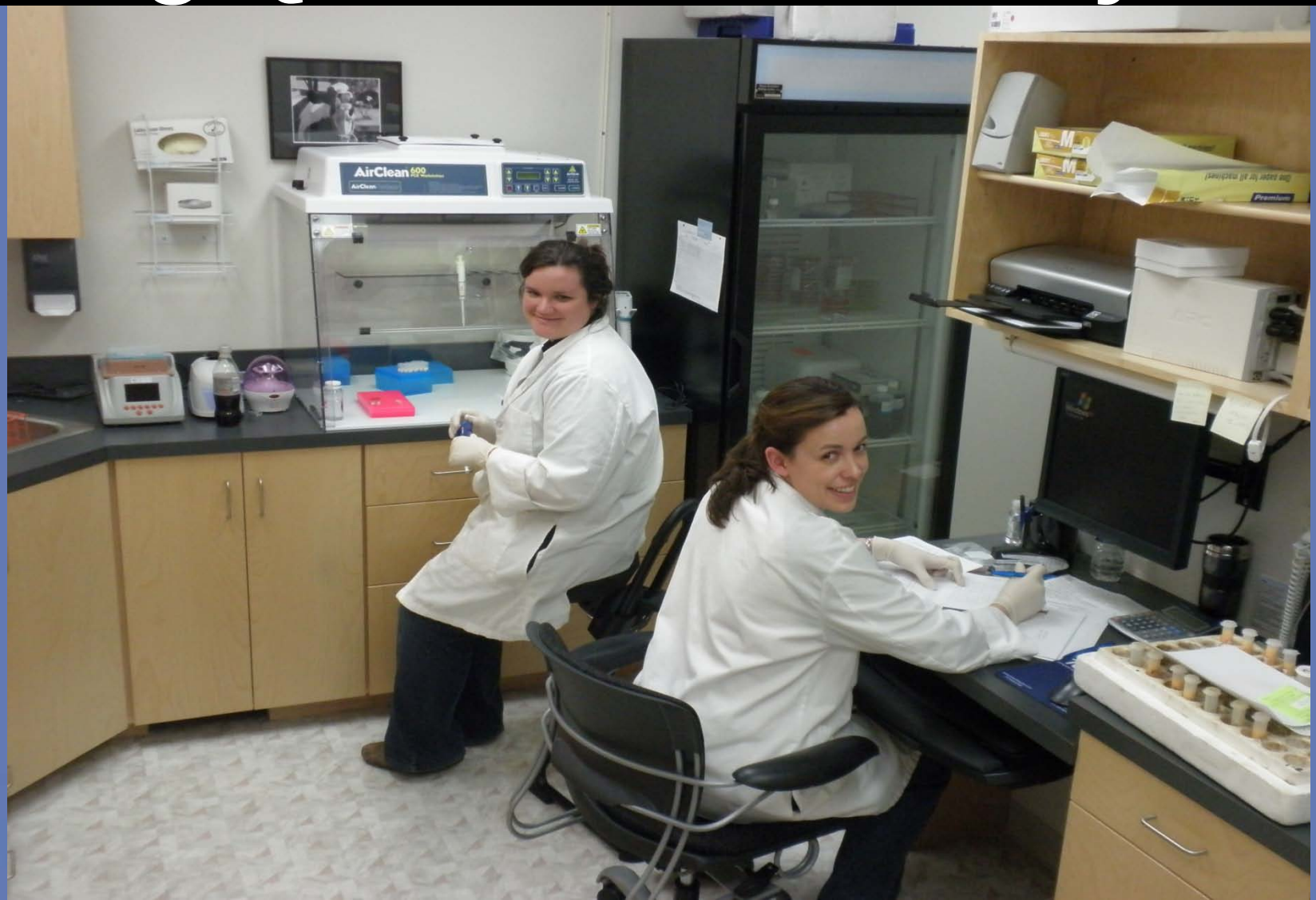
Lancaster DHIA Lab ******* HOT SHEET *******
23001234 45 samples collected 12-31-09 tested 1-05-10

Cows in SCC order Weighted Average SCC: 438

Index	Barn	Milk	Fat	Pro	OS	SCC	Count	DIM	Lac	CAR	W/O	%
4	4	42.0	4.3	3.5	5.4	9.0	6400	232	2		338	24.2
159	159--J	51.0	5.5	4.1	5.9	7.9	2986	272	1		283	13.7
48	48	38.0	4.6	3.7	5.3	7.9	2986	361	4		240	10.2
158	158	67.0	4.3	3.3	5.9	6.1	857	198	1		222	5.2
14280	14--280	67.5	4.3	3.3	5.8	6.0	800	295	1		205	4.9
125	125	79.0	5.2	3.2	5.9	5.7	650	8	2		189	4.6
333	333	47.0	3.3	3.1	4.7	6.4	1056	425	4		170	4.5
165	165	33.0	4.1	3.3	5.5	6.7	1300	609	1		152	3.9
8	8	94.0	3.9	2.8	6.0	4.9	373	109	3		142	3.2
18	18	63.5	4.4	3.4	5.5	5.3	492	332	2		131	2.8
13	13	18.0	4.2	3.4	5.6	7.0	1600	35	2		117	2.6
33	33	97.0	3.8	2.6	5.3	4.1	214	51	5		112	1.9
365	365	32.5	4.0	2.8	5.3	5.6	606	325	1		103	1.8
50	50	61.5	3.0	3.1	6.0	4.6	303	129	3		96	1.7
11	11	45.5	4.5	3.5	5.5	4.9	373	332	2		88	1.5
48261	48--261	79.0	3.7	3.3	5.9	4.1	214	327	2		82	1.5
166	166	75.0	4.5	3.7	5.8	3.9	187	173	2		77	1.3
2234	2234	55.0	3.6	3.0	5.4	4.3	246	224	5		71	1.2
99	99	36.0	5.1	4.0	5.1	4.6	303	336	2		65	1.0
137	137	63.0	3.6	3.3	5.7	3.7	162	429	2		60	0.9
4236	4236	18.0	5.9	3.1	5.2	5.3	492	21	3		55	0.8

Samples come in all shapes and forms (DHIA & Sterile)

5. DNA Lab analyzes sample using QPCR-based analysis



Extracted Mastitis DNA

6. REPORTS

Mastitis PCR Sample Pooling Report

Lancaster DHIA
1592 Old Line Road
Manheim, PA 17545 Phone: (877) 572-4115 Fax: (717) 664-2911 Email: DNA@LancasterDHIA.com

Customer: John Henry Smith
Address: 123 Short Horn Lane
Phone: 777-525-1234

Herdcode: 12345678
Fax: 777-528-4567 **Email:** MoreMilk@Preg.com

Veterinarian: Dr. Kool Hands
Email: MooCow@CLopen.com
Fax: 777-525-4321

When multiple bacteria targets are detected in a sample the percentage of the most abundant bacteria is reported if its proportion is over 90 %.

QUANTITY (specific to each bacterial target): + Low, ++ Medium, +++ High

Full Panel Analysis (Includes *Staph. aureus*, *Staph sp.*, *Strep. ag.*, *Strep. dysgalactiae*, *Strep. uberis*, *E. coli*, *Enterococcus sp.*, *Klebsiella sp.*, *Serrata marcescens*, *Corynebacterium bovis*, *A. pyogenes* and *P. indolicus*, and *Staphylococcal beta-lactamase gene*)

Date: 12/31/2009 8:58:40 AM

Calibration: 9/28/2009 1:41:09 PM

Instrument: PCR1 (Stratagene Mx3000P/Mx3005P QPCR System)

Sample	Bacterial finding	Quantity	Proportion
pool 1	Str.dysgalactiae Klebsiella sp. (including oxytoca and pneumonia)	++ +++	>99%
pool 2	Staph. aureus E.coli Klebsiella sp. (including oxytoca and pneumonia)	++ + ++	
21 RF	Negative	-	-
21 LF	Staph. aureus	++	
83 RR	Klebsiella sp. (including oxytoca and pneumonia)	++	
83 LR	Negative	-	-
585 LR	Negative	-	-
46 RR	E.coli	++	

Sample	Bacterial finding	Quantity	Proportion	
1	Negative	-	-	628,794,812,470,26
2	Negative	-	-	759,349,532,420,707
3	Negative	-	-	723,146,865,19,198
4	Negative	-	-	832, 288, 893, 1887, 666
5	Staph. aureus	+		37, 705, 547, 88, 144
6	Negative	-	-	4150, 335, 722, 3194, 123
7	Negative	-	-	589, 452, 789, 668, 782
8	Negative	-	-	1189, 649, 1199, 97, 473
9	Staph. aureus	+		2984, 858, 2808, 701, 713
10	Negative	-	-	733, 874, 2415, 688, 613
11	Negative	-	-	10, 913, 27, 938, 5
12	Negative	-	-	17, 917, 866, 945, 982
13	Negative	-	-	885, 907, 931, 910, 919
14	Negative	-	-	915, 949, 15, 18, 911
15	Negative	-	-	916, 817, 20, 968, 973
16	Negative	-	-	950, 857, 878, 962, 768
17	Negative	-	-	4063, 596, 5307, 813, 4521
18	Negative	-	-	29, 676, 605, 835, 584
19	Staph. aureus	+		685, 627, 660, 760, 1061
20	Negative	-	-	779, 963, 834, 974, 750
21	Negative	-	-	790, 1885, 3084, 460, 802
22	Negative	-	-	510, 698, 719, 839, 652
23	Negative	-	-	758, 824, 827, 337, 738
24	Negative	-	-	819, 2613, 807, 562, 775
25	Negative	-	-	777, 502, 654, 720, 804
26	Negative	-	-	83, 21, 569, 763, 700?
27	Negative	-	-	826, 1695, 880, 875, 990

Sample	Bacterial finding	Quantity	Proportion
37	Negative	-	-
705	Negative	-	-
547	Negative	-	-
88	Negative	-	-
144	Staph. aureus	+	
7984	Negative	-	-
858	Negative	-	-
2808	Negative	-	-
701	Staph. aureus	suspect	-
713	Negative	-	-
685	Negative	-	-
627	Negative	-	-
660	Negative	-	-
760	Staph. aureus	+	
1061	Negative	-	-

Sample	Bacterial finding	Quantity	Proportion
768	Enterococcus sp. (including faecalis and faecium)	++	
	Klebsiella sp. (including oxytoca and pneumonia)	++	
	E. coli	+	
	Staphylococcus sp.	+	
4063	A. pyogenes and P. indolicus	+	
	Enterococcus sp. (including faecalis and faecium)	+	
	Klebsiella sp. (including oxytoca and pneumonia)	+	
	Staphylococcus sp.	+	
410	Staphylococcus sp.	++	>90%
	Enterococcus sp. (including faecalis and faecium)	+	
596	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	
5307	Staphylococcus sp.	+	
813	Beta-lactamase gene	+	
	Staphylococcus sp.	+	
913	A. pyogenes and P. indolicus	+	
	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	
4521	Beta-lactamase gene	+	
	Enterococcus sp. (including faecalis and faecium)	+	
	Staph. aureus	+	
	Staphylococcus sp.	+	
29	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	
676	Enterococcus sp. (including faecalis and faecium)	+	
	Staphylococcus sp.	+	

Classification	Bacteria	Contagious or Environmental	Source	Spread	Control	Treatment*
***Staphylococcus* spp.**	*Staph. aureus*	Contagious	Infected udders, hands of milkers	Milking time	Post-dip, DCT[1], segregation and cull if necessary	Label recommendations for broad-spectrum antibiotics, if early lactation – 5-7 d pirlimycin, do not treat chronic infections
	Coagulase (-) staph. & *S lyicus*	Neither	Skin flora & occasionally environment	Infect teat canal from skin sources	Post-dip, DCT	Treat clinical cases (broad spectrum), DCT
***Streptococcus* spp. and *Enterococcus* spp.**	*Strep. agalactiae*	Contagious	Infected udders	Milking time	Milking time hygiene, post-dip, DCT	Label recommendations for broad-spectrum antibiotics
	Strep. dysgalactiae	Contagious and environmental	Infected udders and environment	Milking time & environmental contact	Milking time hygiene, pre- & post-dip, DCT, teat seal	Label recommendations for broad-spectrum antibiotics
	Strep. uberis	Environmental	Environment – early dry period	New IMI[2] during early dry period	Milking time hygiene, pre- & post-dip, DCT, teat seal	Label recommendations for broad-spectrum antibiotics and consider IMM[3] therapy 4-5 d penicillin systemically (3.5 cc/100 lb body weight)**
	Environmental strep & *Enterococcus* spp.	Environmental	Environment	Environmental contact	Milking time hygiene, pre- & post-dip, DCT, teat seal	
Coliform	*Escherichia coli*	Environmental	Bedding, manure, soil	Environmental contact	Cows clean & dry, use of sand bedding, pre-dip, a J5 vaccine	Do not treat local cases. Systemic cases – 2-3 L hypertonic saline IV, followed by oral fluid therapy, NSAID*** and injectable antibiotics
	Klebsiella spp.	Environmental	Organic bedding	Environmental contact	Avoid sawdust & recycled manure, pre-dip, J5 vaccine	
	Enterobacter spp.	Environmental	Bedding, manure, soil	Environmental contact	Cows clean & dry, use of sand bedding, pre-dip, a J5 vaccine	
	Serratia spp.	Environmental	Soil and plants	Environmental contact	Cows clean & dry, pre-dip (no chlorhexidine products)	180-300 ml hypertonic saline IMM infusion
	Pseudomonas spp.	Environmental	Water & wet bedding	Environmental contact	No water use in parlor, no cooling ponds, sand bedding, a J5 vaccine	
	Proteus spp.	Environmental	Bedding, feed & water	Environmental contact	Not much known, use of sand bedding, a J5 vaccine	
	Pasteurella spp.	Probably contagious	Upper respiratory tract of mammals and birds	Unknown – likely cow to cow	Prevent teat injuries, remove affected cows from herd	Do not respond to IMM treatment
Other	Yeast & mold	Environmental	Soil, plants, water	Dirty infusions	Aseptic infusions	No treatment
	Corynebacterium bovis & other coryneforms	Contagious	Infected udders	Cow to cow	Post-dip	Treat clinical cases and DCT
	Prototheca	Environmental	Soil, plants, water	Dirty infusions, infected udders	Aseptic infusions, eliminate infected cow	No treatment – cull cow
	Bacillus spp.	Environmental	Soil, water, air	Dirty infusions	Aseptic infusions	Broad-spectrum antibiotic
	Arcanobacterium pyogenes	Contagious/Environmental	Teat injuries	Flies	Fly control	Kill affected quarter or remove from herd

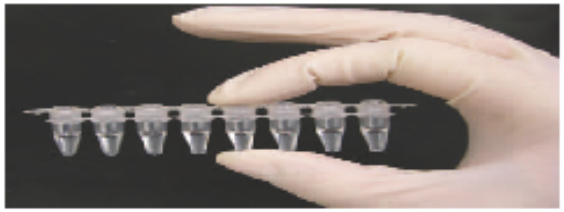
Information obtained from NMC Laboratory Handbook on Bovine Mastitis and veterinary consultation for treatment recommendations).
*These are general treatment recommendations; actual recommendations may vary from herd to herd. Please consult your veterinarian.
**Extra label usage; please consult your veterinarian before starting this protocol.
***Nonsteroidal anti-inflammatory drugs.
[1] – DCT, dry cow therapy; [2] – IMI, intramammary infection; [3] – IMM, intramammary.

C. S. Petersson-Wolfe and J. Currin
Virginia Tech Mastitis & Immunology Laboratory &
Virginia Maryland Regional College of Veterinary

Costs of PCR-based Mastitis Testing

- Lancaster DHIA Customer Price
  - $24 Full Panel Test
  - $19 Contagious Test
  - Volume Discount over 30 samples
- Non LDHIA Customer Price
  - $26 Full Panel Test
  - $21 Contagious Test
  - Volume Discount over 30 samples
- Group Discounts for Milk Cooperatives
- Shipping available to all areas in USA
- All Pricing Agreements must be approved by Lancaster DHIA management

For questions regarding pricing, shipping and all other inquiries please contact Jere High.



Lancaster DHIA

1592 Old Line Road
Manheim, PA 17545

Phone: 1-888-202-3442
Fax: 717-664-2911
E-mail: Jere@LancasterDHIA.com
WWW.LancasterDHIA.com

410

Lancaster DHIA

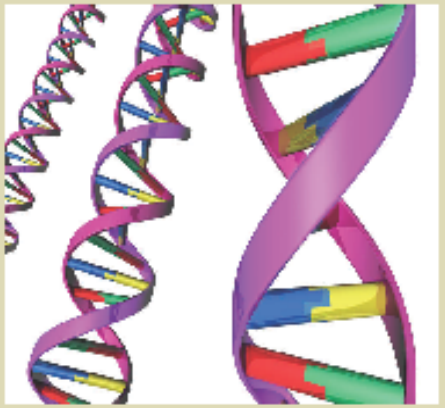
provides the following services:

- **DHIA Milk Testing in PA, NY and Maryland**
  - Nationally Certified
  - On-farm Computer PCDART
  - Average of 2 day turn-around time
  - Call 1-888-202-3442
- **Microbiology Lab Services include**
  - PA State Certified
  - Dairy Water testing for E-coli & Coliform
  - PA Raw Milk Permit Testing
  - IMS Finished Product Testing
  - Call 1-877-750-7058
  - Janice@LancasterDHIA.com
- **Culture Lab Services include**
  - Milk, Hand Towel & Bedding Cultures
  - USDA Certified Johne's Milk ELISA Testing
  - Culture@LancasterDHIA.com
- **DNA Lab Services include**
  - PCR-based DNA Mastitis Testing
  - Call 1-877-572-4115
  - DNA@LancasterDHIA.com

Lancaster DHIA

WANTED

DEAD OR ALIVE



MASTITIS DNA

Reward

- Improved Treatment
- Improved Milk Quality
- Improved Milk Production

PCR-based DNA Mastitis Testing Now Available

- **What is PCR-based Mastitis Testing?**

PCR is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of a particular DNA sequence.

The real-time PCR-based mastitis assay is a revolutionary method for cow mastitis testing. The assay can identify and quantify 11 major mastitis-causing species or groups plus Staphylococcal β-lactamase penicillin resistance gene.

- **What are the advantages?**
  - Samples do not need to be sterile, but can be taken aseptic and shipped in.
  - The preserved samples from Lancaster DHIA testing can be used
  - You don't need to collect a separate sample
  - You can also pool samples from groups of cows to help cut down on costs.

Other advantages include selecting only your high SCC cows after they have been tested in the Lancaster DHIA lab. You can also tell your technician that you want all cows over a certain level of SCC tested. Plus, you can request testing of any fresh cows from the past month to help prevent a new case of mastitis.

We will send results to both you and your veterinarian aiding in more efficient treatment and decreased milk loss.

- **Why use PCR-based Mastitis testing over the standard culture?**

In approximately 25–40 % of bovine milk samples taken from animals with clinical mastitis, no bacterial growth can be detected in conventional culturing.

PCR-based mastitis testing leads to more effective treatments by:

- More specifically identifying coliforms
- Identifying sources of mastitis in a single cow or bulk tank sample
- Identifying sources of mastitis in cows already being treated
- Decreasing test time from 2-10 days (conventional culturing) to 1-2 days from when we receive the sample at the lab.
- Finding Sub-clinical cases before they become clinical or chronic

- **How do I get started?**

You can ask your Lancaster DHIA technician on test day or call the office. If you are not a Lancaster DHIA customer, please call and we can arrange to have sample vials sent to you.

This is the first Real-Time PCR-based DNA Mastitis Test offered in the USA

- **What Tests are performed?**

You may send a bulk tank sample or individual cows for 2 different tests. Listed below are the specific tests for each analysis.

Full Panel Analysis

Contagious Pathogens

1. *Staphylococcus aureus*
2. *Streptococcus agalactiae*

Environmental Pathogens

3. *Corynebacterium bovis* *(Contagious in some cases)*
4. *Streptococcus dysgalactiae (Contagious also)*
5. *Staphylococcus sp.* *(including all major coagulase-negative staphylococci)*
6. *Streptococcus uberis*
7. *Escherichia coli*
8. *Enterococcus sp.* *(including E. faecalis and E. faecium)*
9. *Klebsiella sp.* *(including K. oxytoca and K. pneumoniae)*
10. *Serratia marcescens*
11. *Arcanobacter pyogenes and Peptoniphilus (Peptostreptococcus) indolicus*

Miscellaneous – Linked to staph species

12. *Staphylococcal β-lactamase gene*

Contagious Analysis

1. *Staphylococcus aureus*
2. *Streptococcus agalactiae*
3. *Mycoplasma bovis*

“I have been using the Mastitis PCR test to help my producer’s reduce their PI counts. Strep uberis and Strep dysgalactiae mastitis both shed bacteria which are picked up by the PI test. The new shipping packets and quick turn around by Lancaster DHIA on the PCR test allow me to provide answers in regards to whether my producer’s PI issues are being caused by mastitis in a timely and convenient manner.”

Jim Hassinger

Area Procurement Supervisor, Land O’Lakes, Inc.

7. Work with your veterinarian

THANK YOU

& Questions