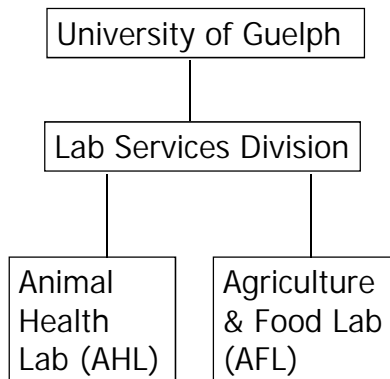


Laboratory Diagnosis and Investigation of *Mycoplasma bovis* at the Animal Health Lab, University of Guelph

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Expertise

16 Veterinarians (microbiologists/pathologists)
~80 technical staff

Diagnostic Cases (per year)

> 400 tests available
> 45,000 cases
> 750,000 procedures

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Mycoplasma Lab at the AHL

- Two full time technicians (>30 year experience)
- Culture isolation/identification
- Serology test
- Tissue slide FA test
- PCR assays
- Antimicrobial susceptibility test

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M. bovis Isolation/Identification

- Isolation

- Haylick's agar plate and broth

- Identification

- Fluorescent antibody test

Routine antisera

M. bovis

M. canadense

M. bovis

M. arginini

A. laidlawii

M. bovirhinis

Alternative antisera

A. modicum

M. verecundum

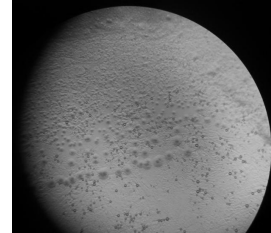
M. sp. bovine group 7

M. sp. group 8 (M. alkalescens)

M. sp. (California calf)

A. axanthum

M. californicum



- 16S rRNA sequencing

- Challenge: difficult for mixed cultures

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M. bovis Isolation Cases

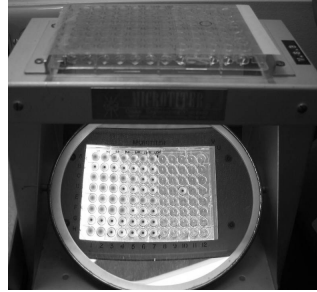
YEAR	<i>M. bovis</i> cultures	positive <i>M. bovis</i> cultures	
1998	177	31	17.5%
1999	278	18	6.5%
2000	223	22	9.9%
2001	453	184	40.6%
2002	332	91	27.4%
2003	255	62	24.3%
2004	218	58	26.6%
2005	166	35	21.1%
2006	136	34	25.0%
2007	264	42	15.9%
2008	330	30	9.1%
Total (11 years)	2832	607	21.4%

Total bovine cases: ~100,000, 0.6% positive of *M. bovis* culture

M. bovis Passive Hemagglutination (PHA) Test

Cho, Ruhnke and Langford (1975)

- PHA titer = 1:80 in 148 out of 200 cow from 4 positive herds
- PHA titer =1:40 in 116 normal control cows



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M. bovis PHA Test

- Re-evaluated by Caswell's group** (Gagea MI et al., 2006)
 - Joint fluid
 - PHA positive: 25 of 28 (89%) cases with arthritis
 - 31 of 70 (44%) cases without arthritis
 - Serum (using culture as gold standard)
 - Relative sensitivity 89%
 - Relative specificity 56%

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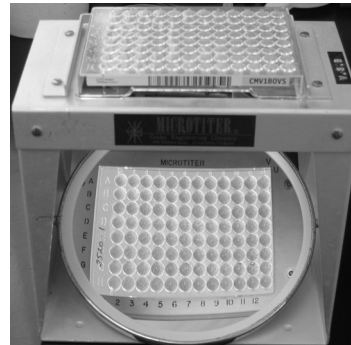
M. bovis PHA Test Cases

YEAR	PHA cases Tested	PHA cases positive	
1998	1	0	0.0%
1999	3	2	66.7%
2000	12	9	75.0%
2001	120	80	66.7%
2002	65	50	76.9%
2003	35	30	85.7%
2004	18	16	88.9%
2005	15	9	60.0%
2006	17	15	88.2%
2007	11	7	63.6%
2008	7	0	0.0%
Total	304	218	71.7%

M. bovis Antimicrobial Susceptibility Test

- PPLO broth + 5% almarBlue
- 37°C, 96 hours
- Blue: inhibition (no growth)
- Challenge: need to order min 500 plates/\$15,000 with 1 year shelf life

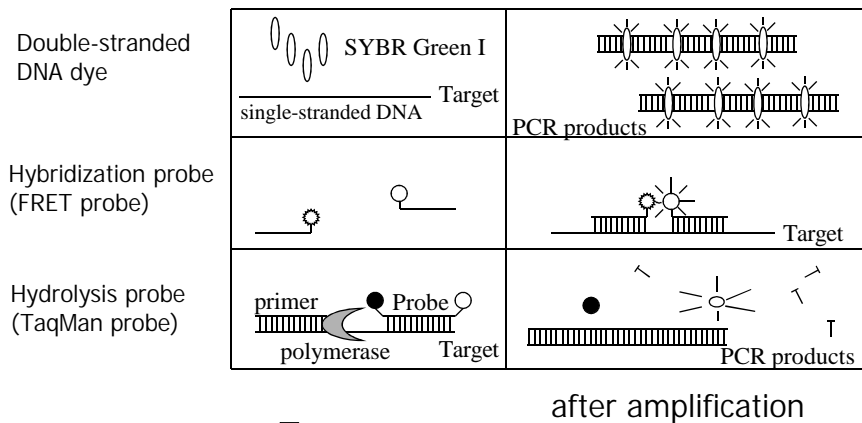
(Rosenbusch et al., 2005)



M. bovis real-time PCR, a better diagnosis tool?

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Real-time PCR Chemistry



M. bovis real-time PCR Primer/Probe

<i>A. pyogenes</i>	M29552	(350)	GAGGCAGCAGTGGGAAT	(412)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(505)	CGACGCCCGGTAATAC
<i>E. coli</i>	280724	(339)	GAGGCAGCAGTGGGAAT	(401)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(512)	CGACGCCCGGTAATAC
<i>X. poeppelii</i>	AY369139	(303)	GAGGCAGCAGTGGGAAT	(365)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(476)	CGACGCCCGGTAATAC
<i>S. marcescens</i>	AB117954	(327)	GAGGCAGCAGTGGGAAT	(389)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(500)	CGACGCCCGGTAATAC
<i>M. morganii</i>	AF549398	(315)	GAGGCAGCAGTGGGAAT	(377)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(488)	CGACGCCCGGTAATAC
<i>M. hemolytica</i>	M75063	(346)	GAGGCAGCAGTGGGAAT	(408)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(519)	CGACGCCCGGTAATAC
<i>P. multocida</i>	AY078997	(327)	GAGGCAGCAGTGGGAAT	(389)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(500)	CGACGCCCGGTAATAC
<i>P. aeruginosa</i>	AB119535	(315)	GAGGCAGCAGTGGGAAT	(377)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(488)	CGACGCCCGGTAATAC
<i>L. interogenus</i>	212817	(283)	GAGGCAGCAGTGGGAAT	(345)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(442)	CGACGCCCGGTAATAC
<i>T. monocytogenes</i>	AF535701	(283)	GAGGCAGCAGTGGGAAT	(355)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(466)	CGACGCCCGGTAATAC
<i>S. aureus</i>	AF146368	(313)	GAGGCAGCAGTGGGAAT	(375)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(485)	CGACGCCCGGTAATAC
<i>S. capitis</i>	BD009397	(348)	GAGGCAGCAGTGGGAAT	(410)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(521)	CGACGCCCGGTAATAC
<i>S. epidermidis</i>	DF83563	(332)	GAGGCAGCAGTGGGAAT	(392)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(503)	CGACGCCCGGTAATAC
<i>S. hominis</i>	U37501	(328)	GAGGCAGCAGTGGGAAT	(390)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(501)	CGACGCCCGGTAATAC
<i>S. agalactiae</i>	AB023574	(327)	GAGGCAGCAGTGGGAAT	(389)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(501)	CGACGCCCGGTAATAC
<i>S. agalactiae</i>	AF015928	(327)	GAGGCAGCAGTGGGAAT	(389)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(501)	CGACGCCCGGTAATAC
<i>S. pyogenes</i>	AF076028	(309)	GAGGCAGCAGTGGGAAT	(371)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(483)	CGACGCCCGGTAATAC
<i>S. uberis</i>	AB023576	(327)	GAGGCAGCAGTGGGAAT	(389)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(501)	CGACGCCCGGTAATAC
<i>S. zooepidemicus</i>	AB104843	(315)	GAGGCAGCAGTGGGAAT	(377)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(489)	CGACGCCCGGTAATAC
<i>S. suis</i>	AF009494	(340)	GAGGCAGCAGTGGGAAT	(402)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(514)	CGACGCCCGGTAATAC
<i>S. intermedium</i>	AF104675	(346)	GAGGCAGCAGTGGGAAT	(408)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(520)	CGACGCCCGGTAATAC
<i>C. perfringens</i>	AB075767	(314)	GAGGCAGCAGTGGGAAT	(376)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(462)	CGACGCCCGGTAATAC
<i>M. alkalescens</i>	AY016348	(56)	GAGGCAGCAGTGGGAAT	(115)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(129)	CGACGCCCGGTAATAC
<i>M. cellulosum</i>	AY760031	(51)	GAGGCAGCAGTGGGAAT	(107)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(129)	CGACGCCCGGTAATAC
<i>M. alkalescens</i>	U94764	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(479)	CGACGCCCGGTAATAC
<i>M. canadense</i>	U04655	(1)	GAGGCAGCAGTGGGAAT	(55)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(166)	CGACGCCCGGTAATAC
<i>M. bovirhinus</i>	U94766	(307)	GAGGCAGCAGTGGGAAT	(369)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. bovisgenitalium</i>	M24291	(344)	GAGGCAGCAGTGGGAAT	(406)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(518)	CGACGCCCGGTAATAC
<i>M. caniflorum</i>	M24582	(370)	GAGGCAGCAGTGGGAAT	(432)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(543)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332743	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332744	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332745	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332746	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332747	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332748	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332749	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332750	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332751	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332752	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. agalactiae</i>	AF332753	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. bovis</i>	AF029255	(304)	GAGGCAGCAGTGGGAAT	(366)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(478)	CGACGCCCGGTAATAC
<i>M. bovis</i>	AF332753	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. bovis</i>	U02968	(344)	GAGGCAGCAGTGGGAAT	(406)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(518)	CGACGCCCGGTAATAC
<i>M. bovis</i>	AF332752	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. bovis</i>	AJ319905	(335)	GAGGCAGCAGTGGGAAT	(397)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(509)	CGACGCCCGGTAATAC
<i>M. bovis</i>	AF332754	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. bovis</i>	AF332755	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC
<i>M. bovis</i>	U94767	(306)	GAGGCAGCAGTGGGAAT	(368)	GATGAAGCCCTATGGGTTGTAACCTCTGTGGT	(480)	CGACGCCCGGTAATAC

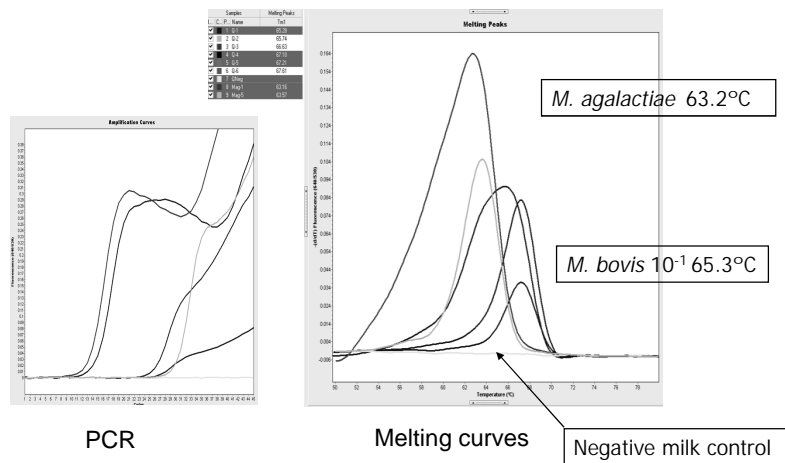
Primer MbsF

Prob Mbs-FL

Probe Mbs-LC

Primer MbsR

M. bovis vs M. agalactiae



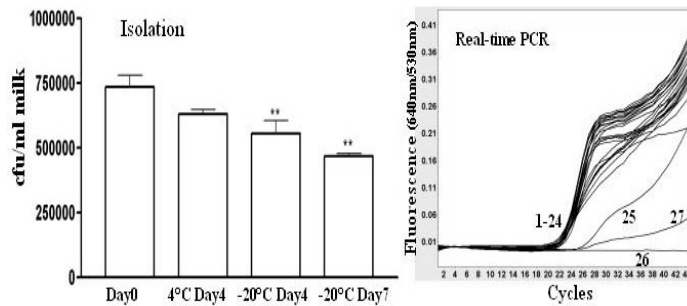
Field Validation: Individual Milks

PCR	Culture		Total
	+ve	-ve	
Positive	13	1	14
Negative	0	151	151
Total	13	152	165

Sensitivity = 100%; Specificity = 93.3%

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Impact of Milk Storage



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M. bovis in Mastitic Milks

Herd level:

Mycoplasma spp. 3% (6/201)

M. bovis : 2.4% (5/201)

Individual milk sample level: 2% (9/440)

Milk with other bacteria >1,000 cfu/mL: 0% (0/114)

Milk with other bacteria <1,000 cfu/mL: 4% (9/226)

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Method Comparison

	Materials+labor(\$)	TAT (d)
Real-time PCR	12+27 _(1h)	0.5
Isolation (negative)	5 +27 _(1h)	10
Isolation (positive)	10+54 _(2h)	3-12

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M. bovis real-time PCR Cases

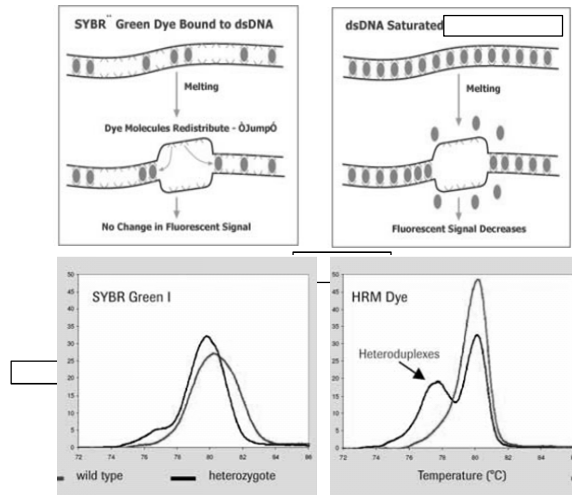
	<i>M. bovis</i> PCR cases	<i>M. bovis</i> positive
2007	2	0%
2008	10	50%
2009	4	0%

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Current Project 1:
High resolution melt (HRM) for
Mycoplasma speciation

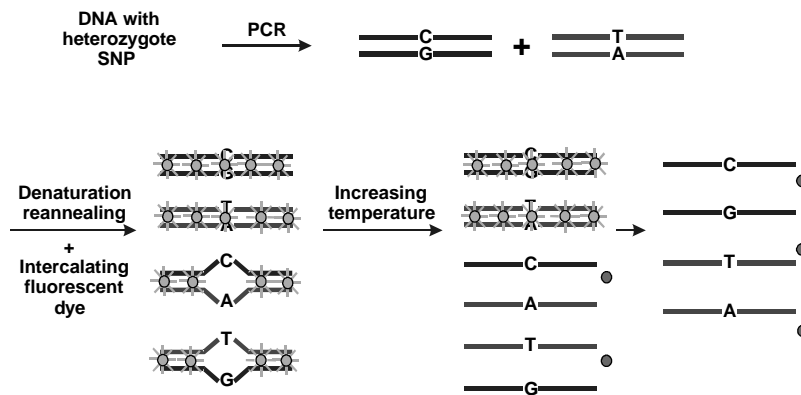
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Melting Curve Analysis



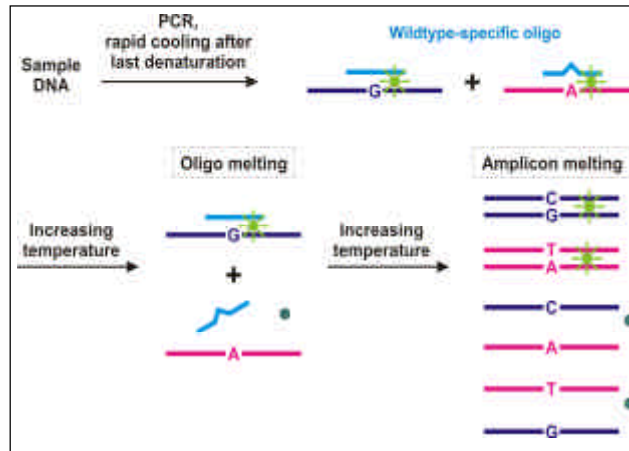
Courtesy Mark Yoshimasu

HRM: Amplicon Melting



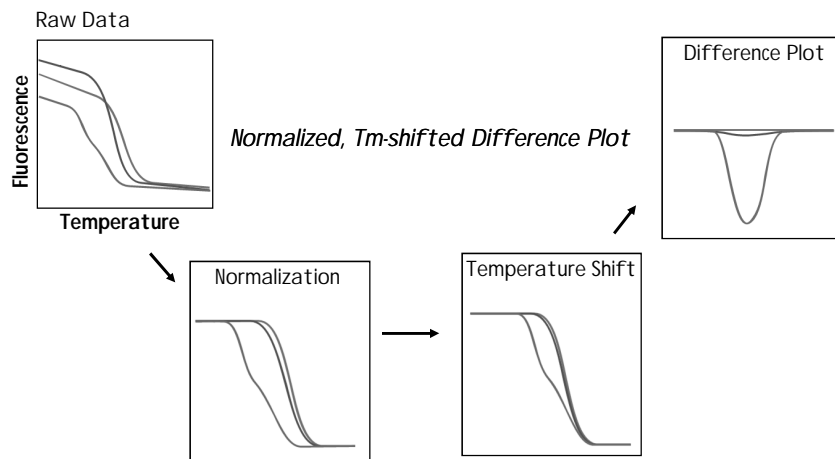
Courtesy Mark Yoshimasu

HRM: Unlabeled Probe Melting



Courtesy Mark Yoshimasu

HRM: Data Analysis

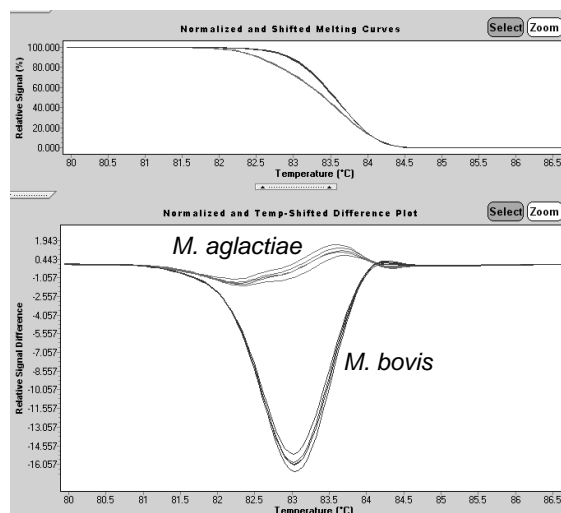


Courtesy Mark Yoshimasu

HRM: Application in Microbiology

- Classification of *M. synoviae* (Jeffery N et al., 2007)
- Identification of clinical bacteria (Cheng J et al., 2006)
- Influenza A subtyping (Lin J-H et al., 2008)
- Classification of fowl adenovirus serotypes (Steer PA et al., 2009)
- Detection and characterisation of infectious bronchitis virus (Steer PA et al., 2009)

HRM: *M. bovis* vs *M. agalactiae*



Current Project 2:
Characterization of *M. bovis*
collected in the last 30 years

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Characterization of *M. bovis* Collection

- AFLP
- Antimicrobial resistance
- Multi-locus sequence typing (MLST)
 - Challenge: *M. bovis* genome sequence not available

Acknowledgments

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Rebeccah Travis
Ana Rita Rebelo

Dr. Beverly McEwen

OMAFRA Animal Health Strategic Investment

Thank you for your attention



New Animal Health Laboratory building-moving in 2010

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