Lessons Learned From The Analysis Of Nucleic Acids In Milk

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AntelBio DHI

THE GLOBAL STANDARD FOR LIVESTOCK DATA

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Milk (Milk Recording) Dirty Diagnostic

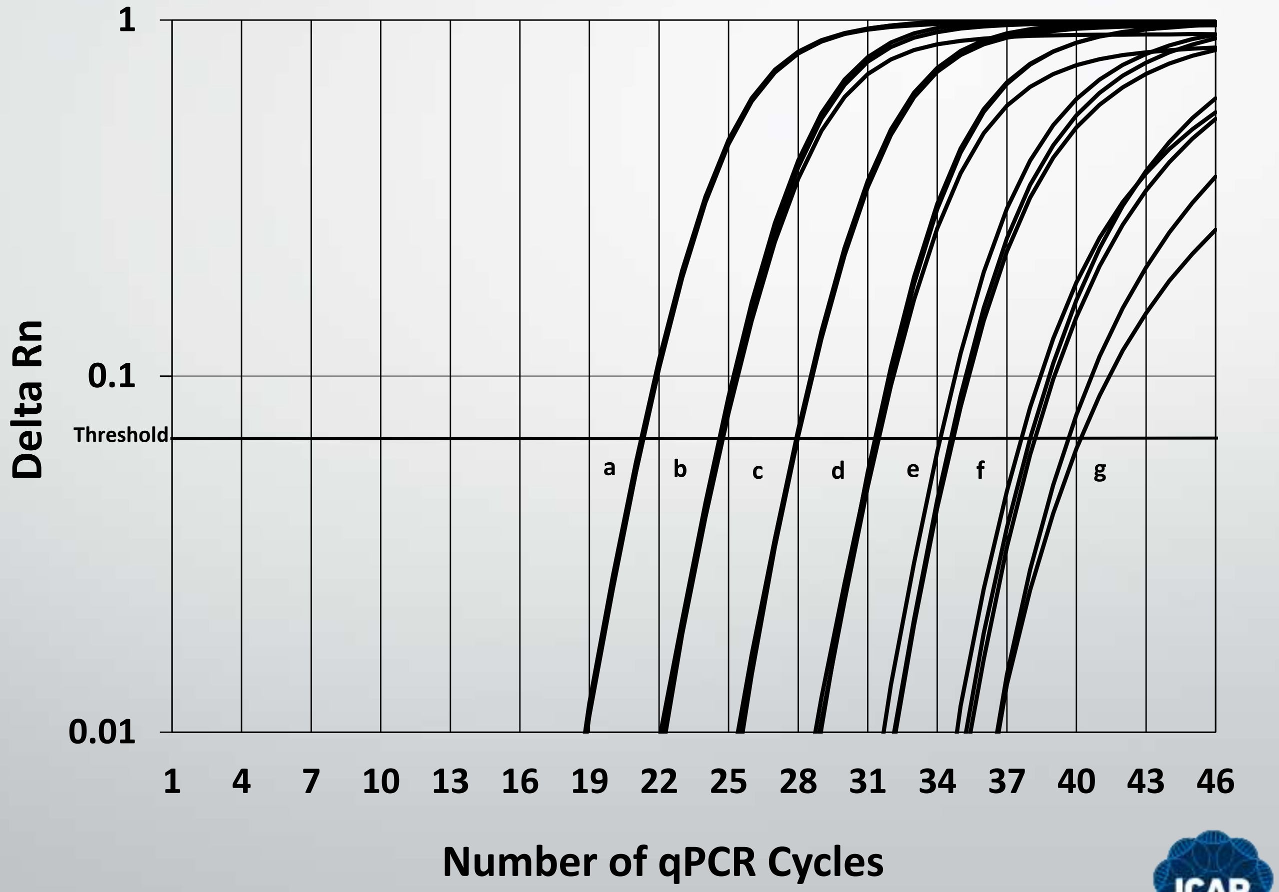
- Historical reluctance to work with milk
  - Food
  - Variable pool size
  - Composition
    - Fat
    - Ca^{2+}
  - Contamination

Commercial Nucleic Acid Tests

- Mycobacteria avium paratuberculosis (MAP, Johne's)
  - Feces
  - Milk
- Bovine Viral Diarrhea Virus
  - Tissue
  - Milk
- Mastitis Pathogens
  - Milk
  - Bedding

Nucleic Acid Analysis

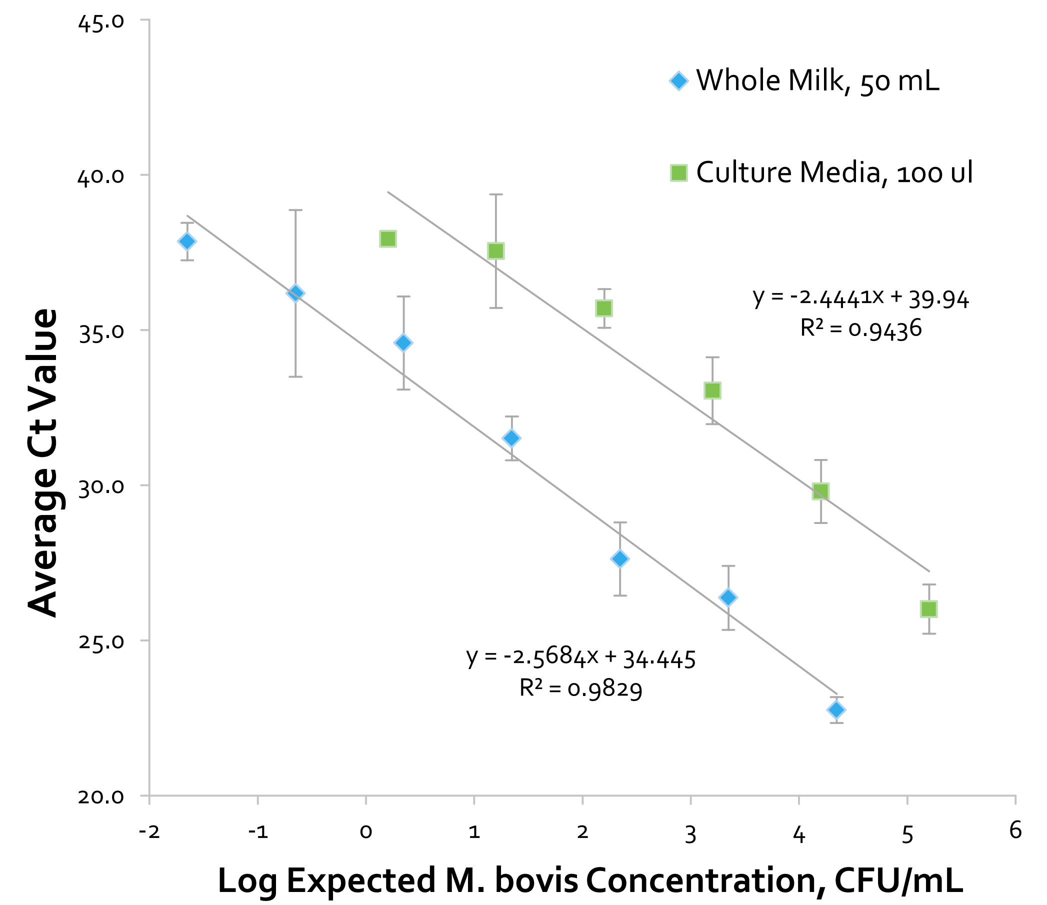
- Real-Time PCR or q PCR (Taqman)



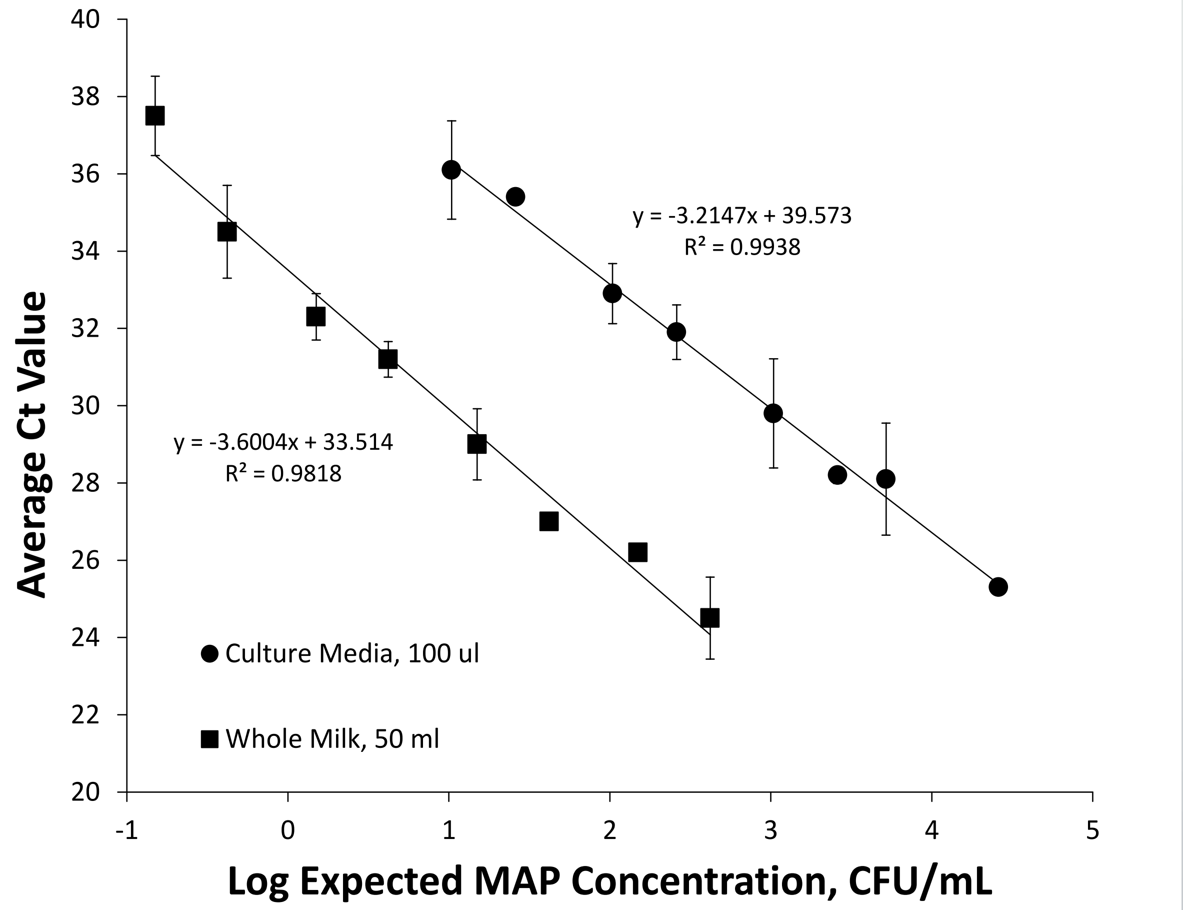
Mycobacteria avium paratuberculosis (MAP) and bovis (TB) qPCR assays

- Bulk Tank Screen

USDA/NVSL
Mycobacterium bovis (TB)



APHIS/NAHMS
Mycobacterium a. paratuberculosis (MAP)



Mycobacterium bovis (TB)

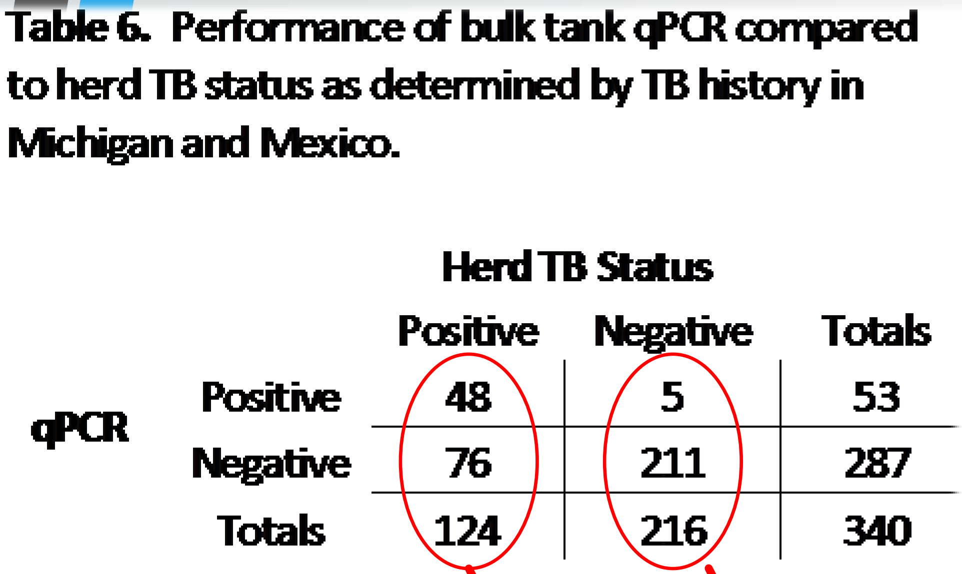


Table 6. Performance of bulk tank qPCR compared to herd TB status as determined by TB history in Michigan and Mexico.

		Herd TB Status		
		Positive	**Negative**	**Totals**
qPCR	**Positive**	48	5	53
	Negative	76	211	287
	Totals	124	216	340

Se=39%

Se=98%

Mycobacterium a paratuberculosis (MAP)

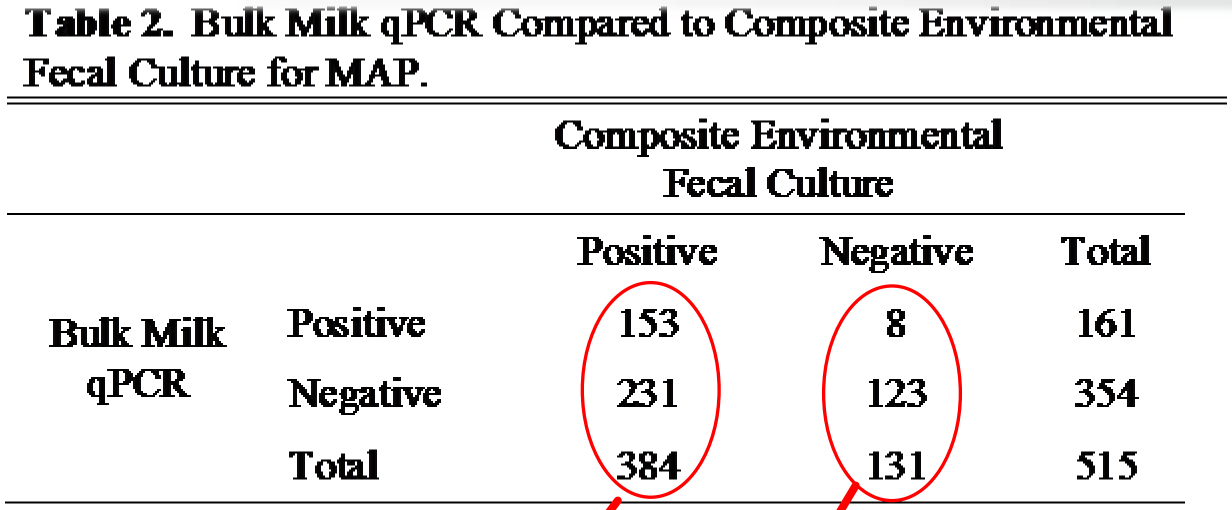


Table 2. Bulk Milk qPCR Compared to Composite Environmental Fecal Culture for MAP.

		Composite Environmental Fecal Culture		
		Positive	**Negative**	**Total**
Bulk Milk qPCR	**Positive**	153	8	161
	Negative	231	123	354
	Total	384	131	515

Se=40%

Se=94%

Individual Animal Samples

Table 7. Comparison of qPCR performance in individual milk samples from cows in TB-positive dairy herds and positive on the caudal fold TB test.

		Animal TB Status		
		Positive	Negative	Total
qPCR	Positive	0	0	0
	Negative	70	0	0
	Total	70	0	70

Where are bulk tank mycobacteria (MAP and TB) coming from?

Parentage Verification

- Animal Identification
- 17% error rate
- Select Sires

SNP Genotyping (Sequenom)

Sample ID	118	1	2	3	…	16	17	…	22	23	24	25	26
AB000001	114		C/C	G/G	A/A	C/C	C/T	C/G	C/T	A/A	T/T	T/T	C/C
AB000002	113		C/C	G/G	A/A	C/C	C/T	C/G	C/T	A/A	T/T	T/T	C/C
AB000007	116	C/C	C/C	G/G	A/A	C/C	C/T	C/G	T/T	A/A	T/T	T/T	C/C
AB000008	114	C/C	C/C	G/G	A/A	C/C	C/T	C/G	T/T	A/A	T/T	T/T	C/C
AB000013	118	C/G	C/C	A/G	A/A	C/C	C/T	C/G	C/C	T/T	C/C	T/T	T/T
AB000014	118	C/G	C/C	A/G	A/A	C/C	C/T	C/G	C/C	T/T	C/C	T/T	T/T
AB000019	117	C/C	C/C	G/G	A/A	C/C	C/T	C/G	C/T	A/T	C/T	T/T	C/T
AB000020	118	C/C	C/C	G/G	A/A	C/C	C/T	C/G	C/T	A/T	C/T	T/T	C/T

Sire Verification

Table 1. Comparison of Sequenom genotyping results between tissue and milk DNA on the 96-SNP bovine parentage panel.

	SNP Count				Correct
Animals[a]	Tissue[b]	Milk[b]	Shared[c]	Discordant[d]	Sire ID[e]
34	3086 (91)	3069 (90)	3089 (95%)	0	34
10	886 (89)	810 (81)	740 (77%)	67 (7)	10
44	3972 (94%)	3879 (92%)	3829 (87%)	67 (2%)	44

[a] Animals were sampled by Typifix ear tags (tissue) and DHIA (milk) in the same week. Animals were divided into 2 categories based on agreement between tissue and milk DNA; absolute (n = 34) and satisfactory (n = 10).

[b] Total calls (average per sample or percent of total) for all available SNPs.

[c] SNP sites (percent of total) with identical results between tissue and milk DNA, including sites that were not called.

[d] For called SNP sites, the number of sites (average per sample or percent total) with different calls.

[e] Number of animals whose sires were correctly verified by milk DNA analysis.

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Sire Discovery

Table 2. Sire discovery[a] with the Sequenom 96-SNP parentage panel in freshened heifers using milk samples or tissue samples (Typifix Ear Tags) in a commercial dairy[b].

		Tissue DNA		
		Yes[c]	No[d]	Total
Milk DNA	Yes[c]	67	12	**79**
	No[d]	11	30	**41**
	Total	**78**	**42**	**120**

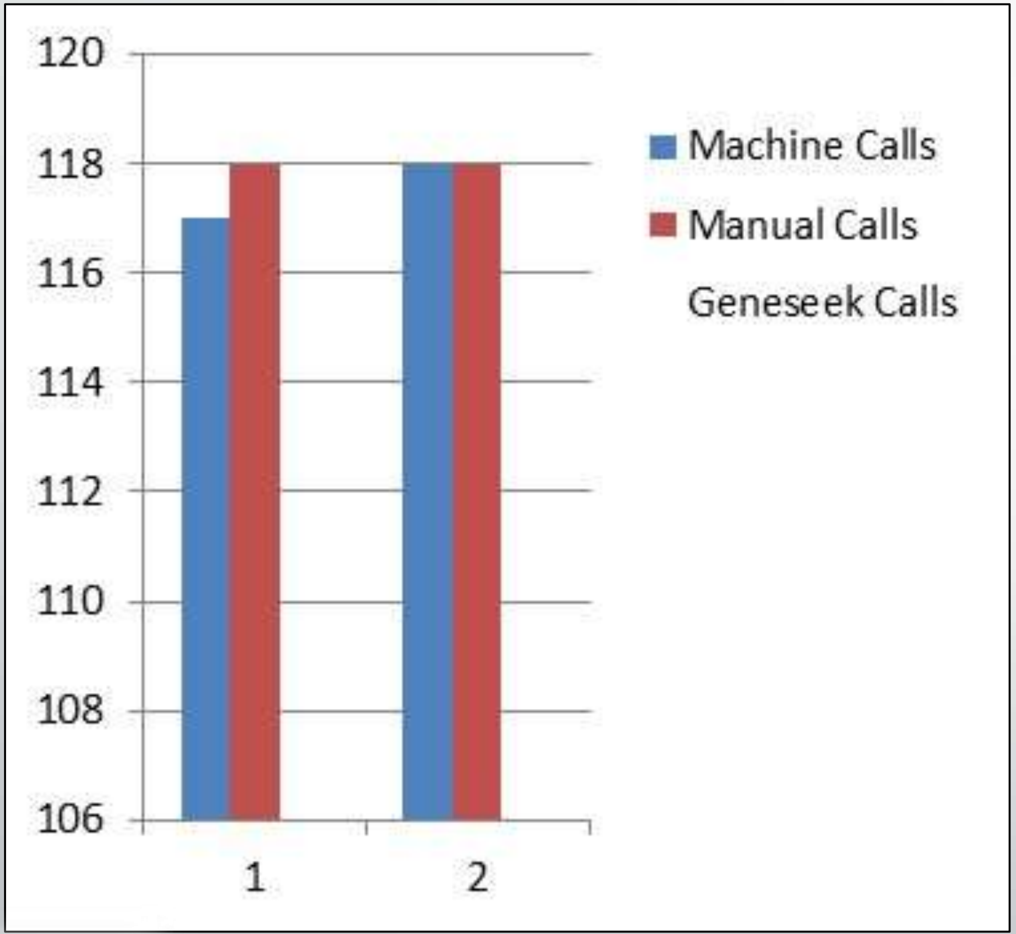
[a] Sire discovery was performed by comparing genotypes from heifers to genotypes from potential sires (n=1034) in the genotyped database.

[b] Inconsistent breeding and heifer records required this PGA herd to use sire discovery to identify likely (>80% probability) sires for genetic evaluation.

[c] A likely sire was discovered in the database using the respective DNA source.

[d] A likely sire was not discovered in the database using the respective DNA source.

Oops!

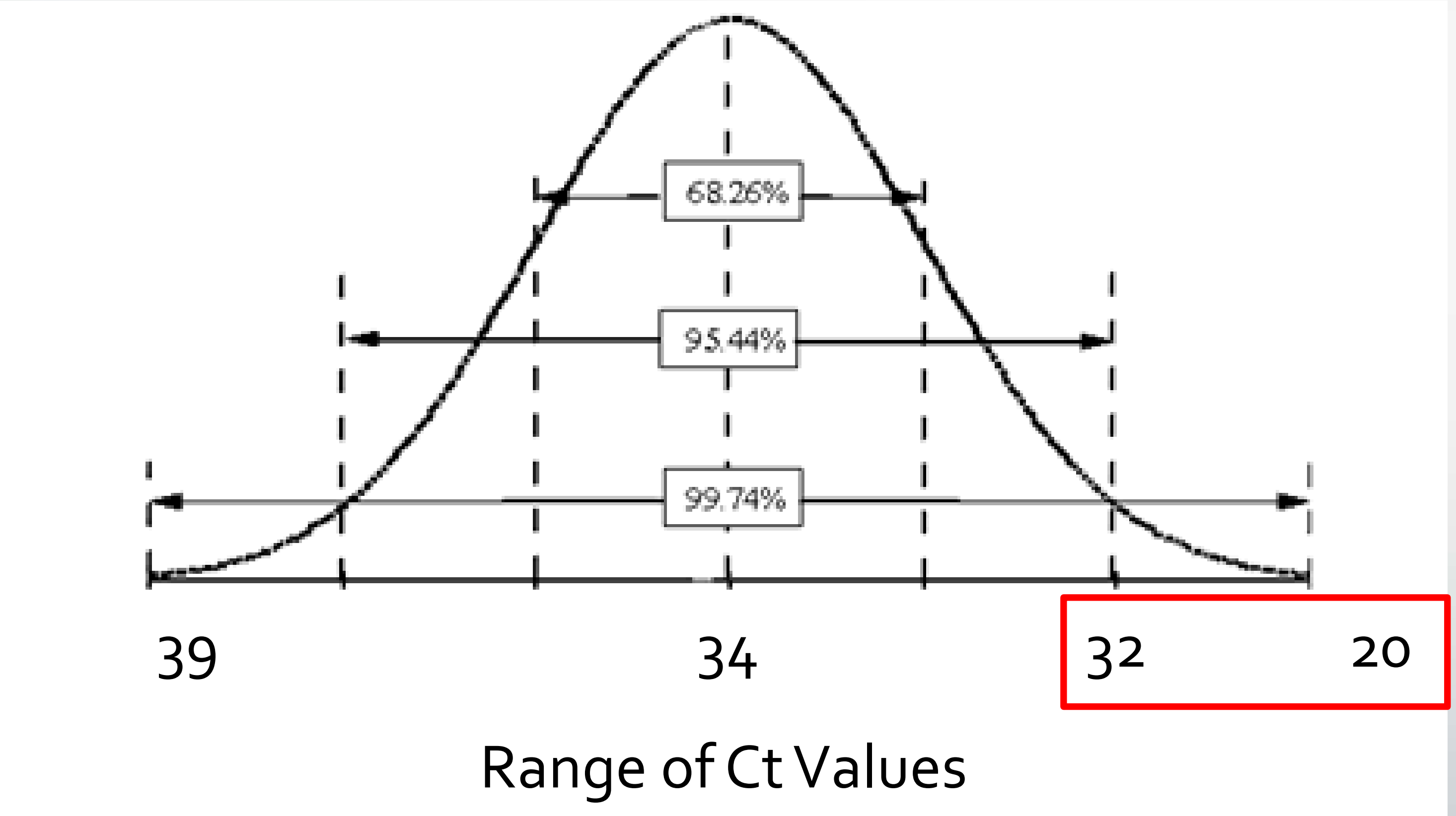


Is milk really a dirty matrix for nucleic acid analysis?

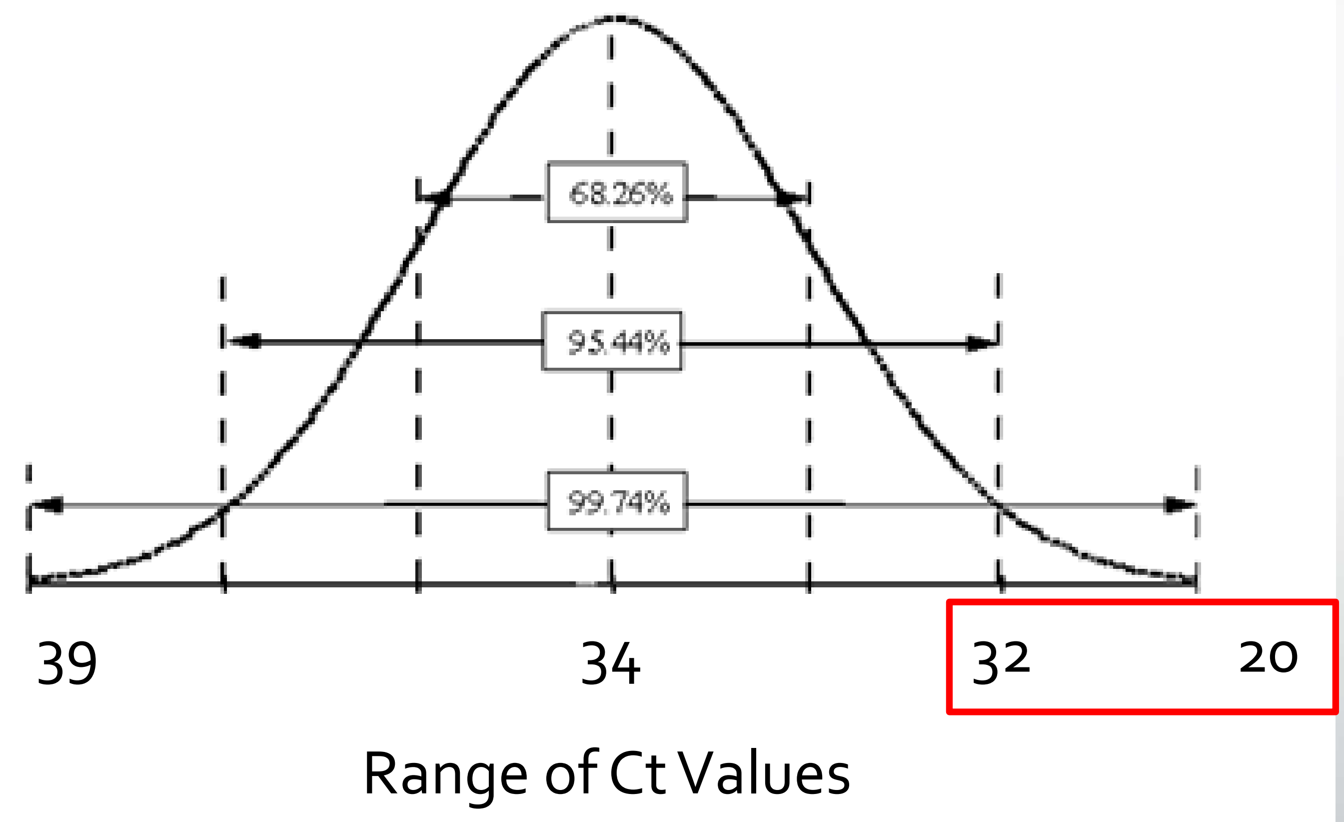
Mastitis PCR for Strep Agalactiae

- Large Dairy
- >10,000 cfu/mL
- Pool DHI sample 5:1 for PCR

Compensating For Carryover



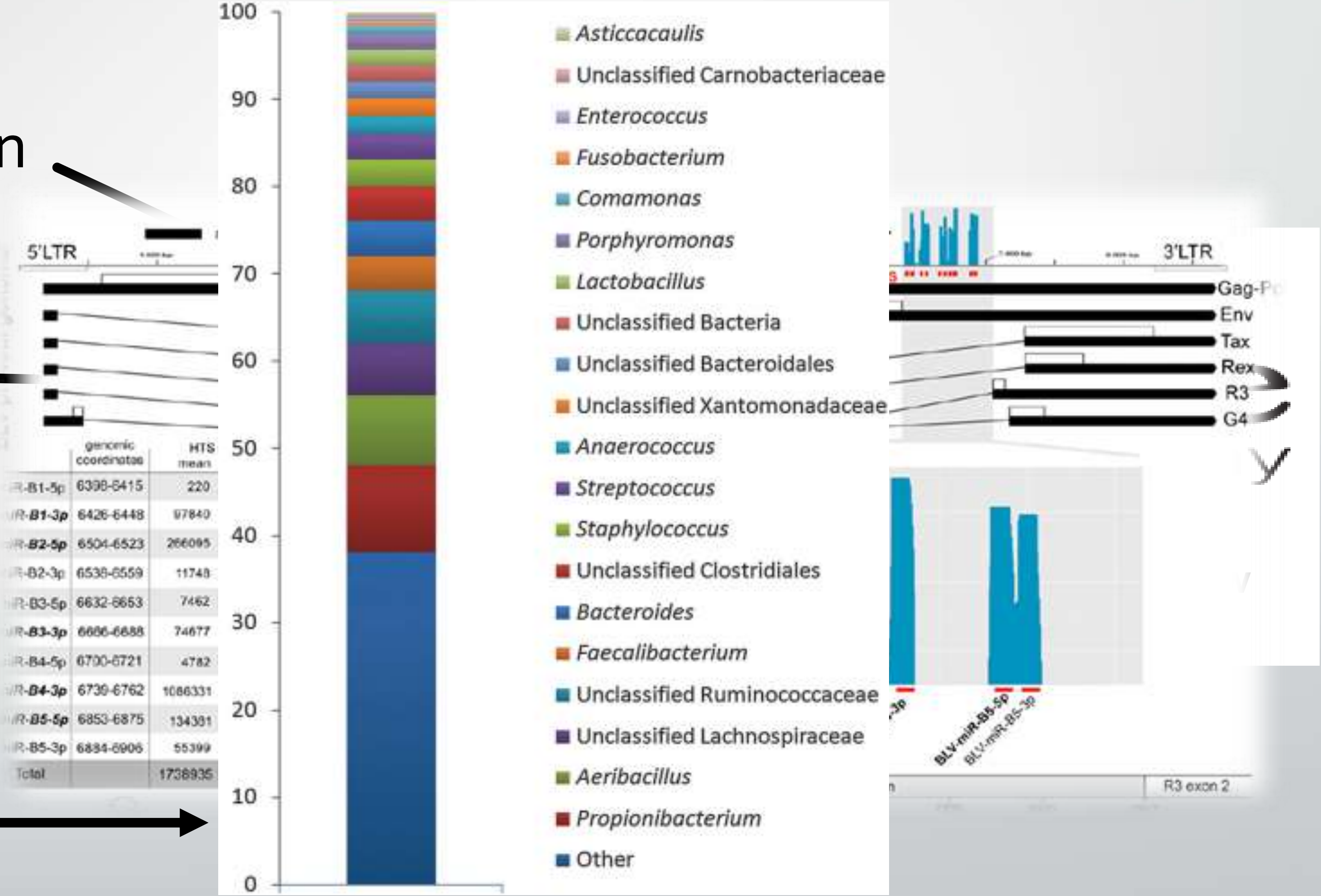
Next Several Bulk Tank Cultures



Is carryover only a number?

Milk Analysis: What's Next?

- Sample ID Verification
- Fetal DNA
- MicroRNA
- Udder Microbiome



	genomic coordinates	HTS mean
R-B1-5p	6398-6415	220
R-B1-3p	6426-6448	97840
R-B2-5p	6504-6523	266095
R-B2-3p	6538-6559	11748
R-B3-5p	6632-6653	7462
R-B3-3p	6666-6688	74677
R-B4-5p	6700-6721	4782
R-B4-3p	6739-6762	1086331
R-B5-5p	6853-6875	134381
R-B5-3p	6884-6906	55399
Total		1738935

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Thank You

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