MILK RECORDING SAMPLES – MULTIPLE ANALYSES, MULTIPLE CHALLENGES

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Carryover – A Significant Cause for Concern

- Carryover is comingling of milk between cows before the sample is tested
- Carryover occurs:
 - Sample collection
 - Cross-contamination in laboratory
- Two effects of carryover:
 - Contamination
 - Dilution





Effect of Carryover in Milk Samples

Cow B Cow A Cow B is correctly screened as negative 1% for Johne's (MAP) Actual data **True Value** 1% Carry-Over Fat % 5.00 Fat % 3.00 **Fat %** 3.02 **Protein %** 3.00 **Protein %** 5.00 **Protein %** 4.98 SCC 1,000,000 SCC 50,000 SCC 59,500 Johne's Johne's Johne's 0 10 1000 Titer Titer Titer

- Minimal carryover effect on fat, protein, SCC
- Would not trigger management actions



Dilution effect in MAP+ samples



MAP Positive Milk Samples

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Johne's ELISA ~ <5% Carryover *not* an issue



Milk Sample Sets

Dilution & Hand stripped sample vs. DHI collected sample studies indicated that 1:20 dilution (5% carryover) would be required to impact ELISA result of the 'Next Cow'.



Effect of Carry-Over in Milk Samples

Cow A

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Cow B

		5%			Co sc, fo	ow B is ind reened as or Johne's	correctly positive s (MAP)
Actual data		, , , , , , , , , , , , , , , , , , ,	True Value			5% Carry-Over	
Fat %	5.00		Fat %	3.00		Fat %	3.10
Protein %	3.00		Protein %	5.00		Protein %	4.90
SCC	1,000,000		SCC	50,000		SCC	97,500
Johne's Titer	1000		Johne's Titer	0		Johne's Titer	100

- Minimal carryover effect on fat and protein
- SCC still would not trigger management actions
- Effect of previous strong+ MAP cow is significant

Leukosis ELISA ~ Carryover a Significant Issue



Dilution studies indicated as little as 1% carryover (1:125) would impact ELISA result of the 'Next Cow' on same milking unit.



Leukosis ELISA Interpretation–Dealing with Carryover



To address carryover - assay protocol included pre-dilution of sample (1:10) and identification of "Suspect" range.



BVD PCR - Carryover at a Whole New Level

- Viral RNA detected consistently from samples diluted 1:1000
- Carryover contamination as low as 0.01% can affect results of subsequent cows
- PCR on DHI samples can be used to identify virus in population of animals
- Follow-up testing (by ELISA) required to identify individuals





S. aureus PCR - effect of milking unit and milking order





Carryover Evidence in S. aureus PCR Samples

Within 2 of PositiveCows

		No	Yes	Total
PCR	Negative	65	12	77
	Positive	6	13	19
	Total	71	25	96

PCR-positive samples affected by carryover:

(by sample position)

8.5% *not* carryover influenced (6/71) <u>52%</u> carryover influenced (13/25)



Effect of Carryover at Herd Level



Possible effects of test false-positive results

- •Culling
- Medical treatment
- Management intervention
- Overestimation of disease prevalence

Investment of valuable resources!

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Carryover in Various Recording Devices

Monthly Meters (usually owned by milk recording organization)

FlaskValve MetersValve Meters with Sampler

Manual sampling via alternating flasks Manual mixing and sampling through valve Direct automatic sampling with/without mixing prior to sampling

Daily Meters (usually installed on the dairy)

•Weigh Jars

•Fill and Dump Meters

•Continuous Flow Meters

Automatic Milking Systems

Total milk collection followed by mixing and subsampling Incremental (cycle) yield measurement with proportionate sampling via manufacturer's device Yield measurement by sensor, proportionate subsampling Direct sampling using external shuttle



Source of Carryover – Visible Residues

Presence of milk from previous cow(s) in:

- Milking cluster
- Hoses
- Milk meter
- Flask
- Sampler



Source of Carryover – Hidden Residues

Hidden residues are a challenge

- Connectors & turns
- Tubes and hoses
- Sampler design
- AMS Pumps
- Environmental
- Laboratory equipment



The Extent of Carryover

• In the Milking Line



150 ml milk remaining in the line Subsequent cow yield of 40lb (18.1kg)

Min. carryover estimate – 0.85% (150ml/17,674ml)

In the Meter Flask



1.5 lbs. milk remaining in flask Subsequent cow yield of 40lb (18.1kg)

Min. carryover estimate – 3.8%



The Extent of Carryover

• In the Sampler



2ml in 25-30ml sample vial Min. carryover estimate – 8-12%

2ml in 80ml sample vial Min. carryover estimate – 2-3%



• AMS Systems



Visible residues? Hidden residues?

Carryover estimates - ???



The Next Steps...

<u>ICAR</u>

- Quantification of carryover occurrence in various milk recording devices
- Standards for allowable carry-over percentage in recording devices
- Guidelines for use of milk samples for health screening tests

Milk Recording Organization

- Develop and implement
 best practices
 - Choice of meter/sampler
 - Equipment maintenance
 - Reduction of visible residues
 - Training of technicians
- Decision tree for suitability of samples for health screening tests





