



## **Assessing the impact of carry-over in routine DHI milk samples when offering additional testing services (Pregnancy, Johne's, Mastitis etc.) through DHI laboratories**

*H. Pearse*

Speaker: Hannah Pearse



# *The impact of carry-over in routine DHI milk samples when offering additional testing services through DHI laboratories*

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RUMINANTS  
WIEDERKÄUER  
RUMINANTS  
RUMIANTES  
反刍动物  
反芻動物

# Herd Recording...

...Is no longer limited to information on Fat, Protein and Somatic Cell Count

- There is enormous value
  - For labs
  - For farmers
  - For veterinarians
  - For industry in general...



...in the additional information that can be obtained from this small milk sample

- Vets and producers now rely on milk test results to make management decisions on metabolic status, infectious disease status, fertility and much more
- But, the results are only as good as the sample that is tested and the test that is chosen to obtain the information

# Carryover Risk in DHI Testing

- There are multiple factors involved in carryover
  - Poorly maintained equipment
  - The size of the sub-sample metered from the cow (dilution factor)
  - The cow's yield
  - Milk line vacuum
  - Milk line hose length
  - The type of milking machine (AMS, meter etc)
  - Technician performance (training to emphasize how to minimize carryover)



## How bad is carryover in the field?

- Study conducted in France to estimate carryover<sup>1</sup> in different milking systems<sup>2</sup>
  - Milk carryover varies significantly depending on the parlour type, but also the set-up of the specific equipment
- Other studies<sup>3</sup> have shown carryover of up to 15% using conventional parlour equipment

Equipment	Milk Volume (ltr)	Range Carryover <sup>1</sup>	Average
Standard AMS	5	8.4-8.5%	8.5%
	8	2.8-3.3%	3.1%
Unadjusted AMS	5	16.9-17.0%	17.0%
	8	19.7-20.0%	19.9%
Well adjusted AMS	6	3.1-4.6%	3.9%
	8	2.1-2.5%	2.3%
Modified AMS	6	10.0-10.5%	10.3%
	8	11.2-11.8%	11.5%
Conventional Parlour	8	3.3-3.7%	3.5%

**BUT – It is widely accepted that carryover levels of less than 5% can be achieved**

# Effect of Carryover on Additional Testing



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# Assays for exogenous components applied to milk recording collected samples\*

Assay	Detection Target	Dilution Detectable	Solution to Carryover
<i>ELISA</i>			
Johne's (MAP)	Antibody	1:20	None required
BLV	Antibody	1:125	Dilution and suspect category
BVDV	Antigen	1:50	None required
Pregnancy (PAG)	Antigen	1:30	None required
<i>PCR</i>			
Johne's (MAP)	Antigen	1:400	Screening, ELISA confirm
BVDV	Antigen	1:10,000	Screening, ELISA confirm
<i>S. aureus</i>	Antigen	1:1,000	Screening, confirm with additional testing or data

\*S.J. Sievert and R.J. Cantin. Best practices to minimize carry-over contamination in milk recording samples, both from operator and from equipment design and set-up perspectives. ICAR, Berlin, 2014

# IDEXX Milk Pregnancy (PAG) Test

- Launched in 2014, the IDEXX Milk Pregnancy Test is the easiest way to detect open cows from 28 days post-breeding and throughout gestation
- Now run in over 100 labs, in more than 30 countries



- >10 Million tests run globally to-date



# IDEXX Milk Pregnancy Test

- Carryover Contamination
  - There is a technical risk of carry-over contamination\* for the IDEXX Milk Pregnancy Test
  - Carryover of <1% does not present a significant risk for false positive or recheck sample
  - 2.5- 5% carryover may increase # of recheck results but low risk of false positives
  - >10% carryover could significantly increase number of recheck and false positive results
- During development, we saw very few 'field-based' issues of carryover contamination
  - >2000 samples tested from routine DHI collections with very few false positive samples that could have been attributed to carryover contamination
- Specificity in field trials and validation testing exceptionally high (>97%)
- Performance of the test could be affected by carryover in specific circumstances
- A high rate of recheck results (>6%) could indicate carryover issues
  - Review % re-check rates on a regular basis (both at a laboratory level and a herd level) to identify potential issues with carryover

\*Data from laboratory-based dilution studies, not field samples

# Mastitis PCR Testing

- Routinely used by herd recording organizations on both DHI and ad-hoc samples to detect infection through identification of bacterial DNA
- Recent studies\* show that carryover of bacterial DNA via the milking unit and milk meter is very likely to affect PCR results for *Staph. aureus*
- Milking order should be considered in mastitis control efforts
- Ct values can be used to classify results and identify potential carryover in DHI samples:

Ct Value	Interpretation
<32	Very likely to be infected with <i>Staph. aureus</i>
32-37	Uncertain <i>Staph. aureus</i> status
>37	Very likely to be negative for <i>Staph. aureus</i>

\*DNA Carryover in Milk Samples from routine milk recording used for PCR-based diagnosis of bovine *Staphylococcus aureus* mastitis. Mahmmod, Klaas and Enevoldsen. J.Dairy Sci 100:1-8 March 2017

## Best Practice – Sampling Checklist

1. Before adding a herd to additional testing services, ensure that the technician has carried out a review of sampling equipment and addresses potential areas of carryover contamination
2. Verify that cow identification systems have been checked for suitability prior to testing
3. Collect samples using an ICAR certified milk meter and ensure that milk sample collection equipment and milking equipment are optimized to reduce carryover
4. Dispense samples into clean vials that contain approved preservative
5. Mix the sample thoroughly to ensure complete dissolution of preservative in the milk
6. Thoroughly drain meter flasks, lines and clusters between samples to reduce carryover contamination
7. Follow best practice process for recording of cow IDs and sample identification to ensure correct assignation of results at the laboratory
8. Modify result interpretation if appropriate and look for indicators that carryover may be affecting results

***If carryover cannot be minimized (<5%) then we need to accept that the farm may not be suitable for additional testing services using routine samples\****

Concentrate on those farms where services can be effective

\*in this instance, hand drawn samples may be substituted to enable access to additional testing services

# Observations

- Additional parameters often require tests with high level of sensitivity to provide relevant diagnostic information
- Manufacturers can implement safeguards from a diagnostic perspective to address the risk of carryover:
  - Recheck zone (IDEXX Milk Pregnancy Test)
  - Interpretation of Ct values in Mastitis PCR testing

***BUT, this is not the final solution***

- In order to achieve high levels of performance with these diagnostic tests, sample quality must not be compromised

# Conclusions

- There is significant value for producers, vets and laboratories in additional testing services
- Long-term success of the herd recording organizations depends on the ability to expand, diversify and add value to the milk sample
- Before enrolling a farm onto any additional testing services, effort must be made to understand, and mitigate the risk of carryover contamination in samples
- Additional testing services are growing throughout the world
- Great work has already been done by ICAR, and the industry as a whole, to better understand carryover
- It is worth the industry continuing this effort to minimize carryover in routine sampling

Thank you!

