Determination of carry-over in automated milking, recording and sampling systems using fluorescent tracers

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Abstract

Integration of yield recording and sampling devices with automated milking systems (AMS) make these more complex with more possibilities for milk residues from one milking to be mixed with milk in the following milking thus creating a carry-over problem. Carry-over can be substantial so that test samples may contain up to 10 percent of milk from the previously milked cow. This study describes the application of a tracer based method for fast determination of carry-over in milk recording equipment under farm or bench conditions. The carry-over tests is based on sequences of milkings of a "phantom cow". In each sequence the first milking is with milk containing a fluorescent color dye as tracer. The milk volume is fixed and samples are taken. The next milking is with pure "white" milk of the same fixed volume. Again, samples are taken using the sampling equipment. The coloring of the white milk in the second sample shows the degree of carry-over. Equipments from leading manufacturers were tested and results showed that carry-over can be a serious problem if any volume of milk is left in containers or pumps. However, with well maintained and adjusted settings for sampling, carry-over reached acceptable values below 4%. In conclusion, the tracer based detection of carry-over is reliable, relatively fast and applicable under field conditions.

Keywords: AMS, carry-over, milk sampling.

1.0 Introduction

The complexity of automated milking systems (AMS) may interfere with recording and sampling units to produce unexpected effects. Rather than testing recorder and sampling units separately these need to be tested as an integral part of the AMS. This has also been the intention with conventional recording and sampling equipment when applying for ICAR approval. However, even devices that have been approved by ICAR may give unacceptable results if used in the complexity of an AMS if not all settings and adjustments are exactly as intended.

One particular problem associated with obtaining milk samples is the carry-over problem where milk from one previously milked cow is mixed with milk from the one currently being milked. The carry-over problem can be rather serious and levels up to 10% were detected by Løvendahl & Bjerring (2006) using a sampler with modifications for scientific purposes.

Methods for detection and estimation of carry-over are not explicitly described in ICAR rules and guidelines. Some indirect measures of carry-over may be the bias and standard deviations but these are not directly interpretable as coefficients of carry-over. Løvendahl & Bjerring (2006) used a statistical approach taking advantage of intensive sampling in a research herd over 14 d periods. The degree of carry-over was estimated as the linear regression of fat percentage in the current sample on the previous sample. Although the method was sensitive it required many samples to become sufficiently powerful, and thereby became expensive.

For purposes of testing newly developed or modified sampling equipment a dedicated carry-over test was developed and validated in the present study. The test is based on use of a fluorescent colour tracer and require approximately 3 hours of down time for running the protocol. Details and validation of the method is reported here.

2.0 Materials and Methods

2.1 Principle

The fluorescent tracer carry-over test use milkings of a "phantom cow", first with a fixed volume of coloured milk and follow that with one or two phantom milkings with similar volume of un-coloured white milk. The coefficient of carry-over is estimated from the colouring of the white milk, using fluorometry. We have used 6 to 8 replicate rounds to ensure consistency of results.

2.2 Detailed protocol

2.2.1 Milk and Tracers

Approximately 200 L of raw milk was set aside for a complete test. For preparation of tracer 25 kg was weighed out in a container (T) and another 30 kg was kept as white milk (W); both were heated to 39°C using an immersion heater. The tracer was prepared by dissolving 600 mg of AY73 (Fluorescein Sodium salt, Sigma-Aldrich, Fluka 46960) and 600 mg of 4MeU (4-Methylumbelliferon Sodium salt, Sigma-Aldrich M1508), first in 40 mL of milk and then mixed up in the 25 kg container. The tracer solution was visibly yellow. The chosen tracers are known to mix well with water and lipids, and have no specific affinity towards plastics or other surfaces more than water and fat itself. From both batches of T and W milk a sample of 300 mL was taken to be used for preparation of calibrators in the lab. The milk was kept warm until used mainly by covering containers with lids.

2.2.2 Phantom cows

Two phantom cows were each constructed from containers holding at least 10 L and a set of 4 plastic tubes (i.d. 4 mm, L = 2 m) ending in hard plastic "teats" with 4 mm openings. One cow was the "Yellow" tracer cow and the other the "White" cow.

Each phantom cow was assigned to an ID-tag used by the AMS to identify it and trigger the milking process.

2.2.3 AMS settings

The AMS were set in "recording mode" whatever that involved of mixing and extended pumping time and mounting of T-pipes.

The line leading milk from AMS to bulk tank was dismounted and diverted into a collection container. In some cases diversion of milk to "drain" was used instead, but milk was always collected in a container and weighed.

2.2.4 Samplers

The method was applied to various makes of samplers as delivered or following deliberate modifications for research purposes. Also, a recorder and sampler unit for use in parlours was tested using this method. All samplers were set to give 24 mL standard samples.

The method has been applied to a number of different equipments and some examples were included in the present study (Table 1). It should be noted that the current study was only conducted to validate the method and never for comparisons between specific equipments.

Table 1. Milk sampling equipments tested for carry-over.

Test no.	Type	Modifications
1	AMS	Standard
2	AMS	Standard unadjusted
3	AMS	Standard adjusted
4	AMS	Research modified
5	Manual Parlour	Standard electronic meter and sampler

2.2.5 Testing protocol

We have mostly used 6 to 8 replicate runs as that gives sufficient power and fits well with using two batches of tracer. The phantom cow was set to "yield" 5, 6 or 8 kg milk as the carry-over problem gets diluted at higher volumes, and because ICAR states that equipment should reach precision already at low yields.

At each milking, samples (8 mL) for lab-analysis were collected as pre-milking (A) from the phantom cow and following the milking from the sampler-unit (B), and finally from the drain line or bulk line (C). Samples were cooled (4°C) until assayed, usually the day after the test. Bronopol was not avoided nor was it used deliberately, but it had no impact on fluorescence readings in normal, double or triple concentrations (T. Larsen, unpublished). All milk was weighed out and collected milk was weighed. Yield record data from the AMS were collected as supplementary data. The protocol is illustrated in the table below (Table 2).

Table 2 Protocol (example, part) for testing of carry-over in AMS milk sampling. Samples A are pre-test, B is the sampler, C is the end-unit or bulk tank line or waste line.

Replicate	*Run*	*Liquid*	*Volume (Kg)*	*Samples*
1	1_1	Yellow	5	A **B** C
	1_2	White	5	A **B** C
2	2_1	Yellow	5	A **B** C
	2_2	White	5	A **B** C
	2_3	White	5	A **B** C
3	3_1	Yellow	8	A **B** C
	3_2	White	8	A **B** C
4	4_1	Yellow	8	A **B** C
	Wash	No		
	4_2	White	8	A **B** C

2.3 Fluorescence determination and calibration

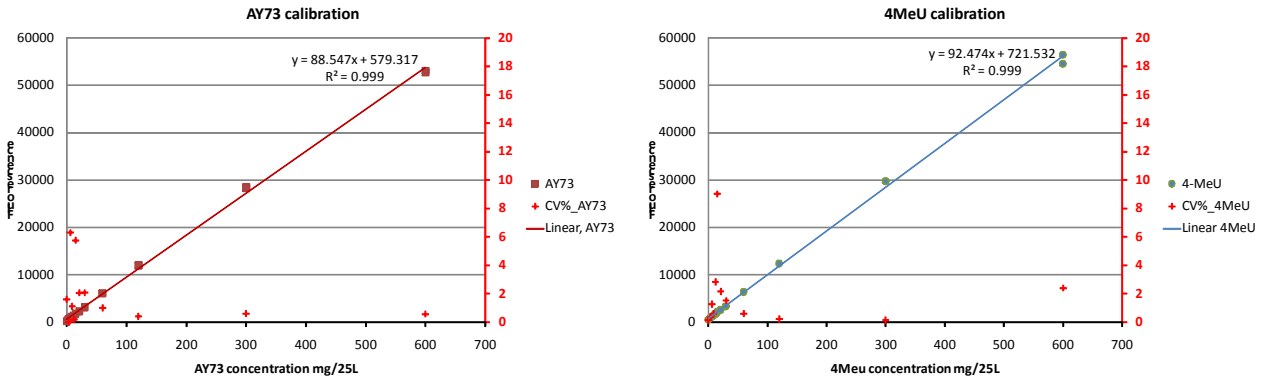
Fluorescence intensity was measured using excitation filter at 485 nm and emission filter at 520 nm for the AY73 and at 355 / 460 nm for the 4MeU. "White" milk readings were always low, but were included as the 0 (zero) calibrator. The "yellow" sample from each batch was defined as 600 arbitrary units, being equal to 100 % colour saturation. Ten more (10) calibrators were initially made for all intermediate values by mixing white and yellow milk. Calibrators and samples were dispensed into duplicate wells of microtitre plates, so that all readings were in duplicate. All calibrators and samples were heated to 39°C before measurement on a fluorometer. Calibration curves were checked for linearity by back-calculating values derived from a simple 2-point calibration. For routine use a 5 point curve was used (0, 10,20, 30, 100 %).

In this setup, carry-over is expressed in units of "yellow" which was proportional to percentage by division with 6.0.

3.0 Results and discussion

3.1 Linearity of Fluorescence Response

Calibration curves for the two fluorochromes are shown below in figures 1 and 2. The fluorescence readings were directly proportional to the concentrations of each tracer over the range used.



Figures 1 (left) and 2 (right). Calibration curves and precision profiles for two colour tracers. Precision is shown as CV% of duplicates (right red axis, + markers).

The linearity of both colours was almost perfect over the range of concentrations and fluorescence readings. The precision profiles showed that duplicate replications was always better than 10% and typically around 2 to 4 % in the range of interest for use, namely between 12 and 120 mg/25L, equal to 2 to 20 % carry-over. "White" milk had a baseline reading of 600 to 700 fluorescence counts, which was deducted from calculated concentrations, using the trend-line as calibration formula. Similar new curves were produced for every batch of tracer and white milk, and used for actual concentrations. In effect, a sample containing 60 units, would have a precision of 2 units or less, equal to a carry-over at 10% ± 0.3 % units, from a single determination.

3.2 Detailed example results

Results are obtained in the form of concentrations in the arbitrary units. For convenience these were immediately recalculated to a scale of 0 to 100% before being presented in table 3 (below). Otherwise results are aligned with the protocol in table 2.

Table 3. Detailed results from a carry-over test on an AMS system. A second order carry-over is shown in run 2_3.

			Pre-sample, A		Sampler, B		End unit, C	
Run	Type	Volume	AY73	4MeU	AY73	4MeU	AY73	4MeU
Carry-over in percent								
1_1	Yellow	5	100.0	100.0	95.1	93.3	91.1	91.0
1_2	White	5	0.4	0.3	18.5	18.0	29.2	29.2
2_1	Yellow	5	100.0	100.0	90.2	93.0	94.1	95.4
2_2	White	5	0.9	0.6	15.4	15.8	18.3	18.7
2_3	White	5	0.5	0.4	2.8	2.8	3.1	2.9
3_1	Yellow	8	100.0	100.0	96.4	98.0	97.8	97.6
3_2	White	8	0.7	0.5	20.0	19.7	18.6	18.3
4_1	Yellow	8	100.0	100.0	95.4	97.4	97.4	99.2
	WASH !							
4_2	White	8	1.0	0.7	1.7	1.4	1.0	0.6
First order carry-over without wash					**18.0**	**17.8**	**22.0**	**22.1**

This example comes from an AMS that required some serious adjustments! However, readings of carry-over are simply found in the lines with "white" milk. There is very good agreement between readings of the two tracers. In the case 2_3 where a second run with white milk was conducted, carry-over was less, but should be seen in relation to the immediate previous sample, and thereby values of around 18% were obtained in B samples. In replicate 4, a short wash cycle interrupted between tracer and white milk. This was clearly effective in removing tracer and residues of milk, leaving practically no signs of carry-over.

3.3 Carry-over in various AMS and samplers

Five different equipments were tested on different occasions, using the same basic protocol but with modifications needed for each special case. Weight was put on the 8 kg results as the lead through all tests. Results are presented in table 4, as extracts from the detailed results from each testing round.

Table 4. Carry-over test results from 5 different setups. Carry-over in percent (CO%) as measured in samples taken from the autosampler.

Equipment	Volume (N)	CO%, AY73	CO%, 4MeU	Average
1.A AMS Standard	5 (1)	8.4	8.5	8.5
	8 (3)	3.3	2.8	3.1
1.B	8 (6)	6.7	7.2	7.0
2 AMS Unadjusted	5 (2)	17.0	16.9	17.0
	8 (1)	20.0	19.7	19.9
3 AMS Well adjusted	6 (1)	3.1	4.6	3.9
	8 (6)	2.1	2.5	2.3
4 AMS Modified	6 (2)	10.5	10.0	10.3
	8 (3)	11.2	11.8	11.5
5 Conventional	8 (6)	3.3	3.7	3.5

Good results were obtained from standard equipment but only when this was well adjusted. These were comparable to results from conventional setups. It is less clear if results are affected by volume of milk. Modifications to samplers may increase carry-over.

4.0 Discussion and Conclusion

4.1 Discussion

This study has presented an effective method of directly detecting carry-over in milk sampling equipment connected to AMS or to conventional parlour milking systems. This method provides fast and precise results and only requires few hours of down-time for the AMS+sampler unit under test. The required chemical tracers are inexpensive and measurements easy to perform in microtitre plates using standard research lab equipment.

Estimates of carry-over based on this method confirmed previous findings of high carry-over in modified samplers for AMS (Løvendahl & Bjerring, 2006).

Visible residues of milk have been detected in AMS units with high carry-over, both in tube connections and in separator and pumping units. Residues of up to 0.5 L were found. These residues were successfully reduced following adjustment of level sensors, and carry-over problems were similarly reduced.

The tests were mostly carried out using 5 to 8 kg of milk in order for the test to be as sensitive as possible, and because sampling should work already from such small volumes. However, most milkings are larger than 10 kg, which will inevitably reduce the problem by simple dilution, as also shown by Løvendahl & Bjerring (2006).

A small degree of carry-over (3.5%) was also detected in the conventional system, and previous findings indicate that at around 2% can be found in another conventional system (Løvendahl & Bjerring, 2006), using normal milkings in a tie-barn system. However, milk volumes were in that case larger and may thereby give lower carry-over.

The described method could be the basis for testing carry-over for approval of combinations of AMS and samplers. The method has a dependency on volume and directions are therefore needed as to appropriate testing volumes, and acceptable levels of carry-over at these volumes.

4.2 Conclusion

The present study has described an efficient method for assessment of carry-over in combined AMS-sampler equipments. This method should be helpful to the manufacturers and to recording organisations for monitoring and improving sampling equipment, and to provide testable tolerances for approval of new or modified equipment.

4.3 Acknowledgements

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5.0 Literature

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