Best practices to minimize carry-over contamination in milk recording samples, both from operator and from equipment design and set-up perspectives.

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Abstract

Carryover contamination (CO) of milk samples is defined as residual milk from one cow that mixes with milk of another cow during sample collection. Considering multiple component samples for a cow over the course of the lactation and a near-random distribution of CO possibilities, the lactation effect on milk fat, protein, and SCC is minimal. With the implementation of new health diagnostic and screening tests, there is a renewed focus on the effect of CO on results. While laboratories performing enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR) tests have developed protocols to minimize the impact of CO, their role is primarily limited to the analysis of the milk sample presented. The role in the reduction of CO of milk samples lies with the on-farm sampling practices and collection, most often by the milk recording organization (MRO) but also from owner sampler herds. The wide range of ICAR-approved recording devices used by MRO to collect representative milk samples vary in design and method of operation, providing varying potentials of CO. Identification of source(s) of probable CO is necessary to impact carryover reduction and provides equipment manufacturers useful information from a design perspective. Acceptance of the limitations of recording devices along with the development of best practices to minimize potential CO by recording device operators can provide milk samples for analysis that accurately represent the animal sampled. For automatic milking and sampling systems, these practices can be implemented through alterations in the system programming and design. However, minimizing CO in the collection of samples via traditional recording devices and external samplers is a challenge as it includes a larger human component, therefore training of MRO and owner sampler staff in best sampling practices is critical. Specific focus on best practices to minimize CO for these mechanical recording devices should be placed on 1) specific equipment set-up and maintenance; 2) understanding the impact of the existing milking system on equipment operation; and 3) proper operator usage. These practices should also include provisions for accurate cow identification, sample to cow identification, sampling order, and potentially chain of custody, resulting in increased accuracy.

Keywords: dairy herd improvement, milk recording, recording devices, carryover, milk sampling

Background and perspective

Herd recording programs have traditionally collected individual cow milk samples for analysis of endogenous components such as milk fat and protein (either reported as true or total protein) and exogenous components such as somatic cells (SCC). These milk samples are

collected using ICAR-approved recording and sampling devices with defined tolerances for yield and milk fat percentage that provide both representative samples and accurate results for these components. Even when properly installed and operated under ideal circumstances, the potential for carryover contamination exists within all milk recording and sampling devices. Mitigating the effect of this carryover in traditional herd recording programs is the fact that multiple milk samples are collected for each individual cow over the course of the lactation. Considering that individual milk samples are potentially collected from different recording devices at each recording day, along with the assumption of a random distribution of errors associated with the sample collection and the component analysis, the net effect of carryover on the cumulative lactation yield of individual milk components is minimal. In fact, the effect of the 2% carryover on a cow's milk sample with breed average milk fat or protein concentration is less than or equivalent to the tolerance of the instrument analysing the sample. Longstanding experience indicates errors resulting from carryover contamination are insignificant and that component results are reliable, providing both accurate data for management decisions as well as genetic predictions.

The increase in the use of the milk recording samples for analysis of additional exogenous components, defined for the present purpose as milk components that are either not present in normal, healthy cows, or whose quantification differentiates disease status or physiological state. The primary assays that have been recently applied to milk recording samples in the U.S. are indicated in Table 1. The majority of the assays being applied to milk recording samples can be divided into ELISA-based or PCR-based technologies. Evaluation of these assays has shown that current levels of carryover contamination affect interpretation of results, with the consequences of erroneous interpretation becoming more costly to the dairy producer. While attempts have been made to moderate the effects of carryover contamination on individual assays, it is clear that there is a lack of a systematic evaluation of both new assays and the level and variation in carryover contamination that occurs during routine sample collection. As newer and more sophisticated technologies are being considered to extract more information from individual animal milk samples, it is critical to consider development of standards and procedures to address the impact of carryover contamination.

Table 1. Assays for exogenous components applied to milk recording collected samples.

Assay	Detection Target	Dilution Detectable	Solution to Carryover
ELISA			
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
PCR			
Johne's (MAP)	Antigen	1:400	Screening, ELISA confirm
BVDV	Antigen	1:10,000	Screening, ELISA confirm
S. aureus	Antigen	1:1,000	Screening, confirm with additional testing or data

This paper does not propose to set standards or guidelines for acceptable carryover limits at this time, but rather describes the need to develop and implement best practices to minimize carryover contamination in milk recording samples by the herd recording organization. The goal of the recording organization should be to provide reliably identified milk samples with both minimal and manageable carry-over contamination to the laboratory for analysis by differing analytical procedures.

Defining carryover contamination in milk recording samples

Carryover contamination that occurs during the milk recording and analytical process is defined as the commingling of milk between cows before samples undergo testing. Carryover has two effects on milk sample components - dilution or contamination. Anecdotal estimates of carryover in the milk recording industry range from less than 2% to 20% depending on the sampling equipment and its operation. Most of the focus is therefore on contamination, especially for exogenous components where detection at any level implicates a physiological or health state. For diagnostic assays, contamination reduces the specificity of the test, resulting in an increase in false positive diagnoses when compared to the analysis of blood or hand-stripped milk samples. Therefore, carryover contamination is a significant concern when testing milk recording samples.

The greatest amount of contamination generally occurs during sample collection as a result of residual milk remaining in the milking equipment as well as metering and sampling devices after a cow finishes milking. This residual milk contaminates the milk sample collected from the cow (or in some cases, two cows) following on the same milking unit. It is logical to focus on minimizing the effect(s) of carryover should be implemented by herd recording organizations to provide reliably accurate and representative milk samples for analysis. These efforts should include sample identification and data recording, equipment installation, maintenance, and operation, as well as the recognition that certain devices may not be suitable for the collection of milk samples for specific analyses.

Carryover contamination may also occur in the laboratory during traditional component (milk fat, protein, and SCC) analysis from the sample stirrers and sampling unit, which may or may not be rinsed between samples. While a small component of carryover contamination, it is important to recognize the potential for a laboratory effect as herd recording milk samples are analysed for these components prior to health or diagnostic screening assays. In the latter case, carryover occurs in sample analysis order, which is most likely different than milking order on individual milking units. Between the sampling and the primary laboratory analysis effects, it is possible that carryover contamination likely involves milk from a minimum of three cows under current sampling and analytical conditions. As new technologies are applied to milk recording samples, all forms of carryover contamination need to be addressed at some level. Although the relative importance between sensitivity (false negative) and specificity (false positive) for individual assays will dictate to some extent the impact of carryover contamination on interpretation, if milk recording samples are to be used for increasing amounts of information, efforts to further minimize or eliminate contamination are justified.

While not a specific practice to minimize the effect of carryover, having measurable standards for cow identification, sample identification, as well as stall location and milking order should be included the standard procedures of the milk recording organization. When

the possibility of a false positive sample exists, a review of the milking order and sample analysis order may identify potential first and second order contamination. By identifying a potential contamination, reporting the positive diagnostic screening result as suspect requiring retesting rather than a positive result to the producer serves the needs of the dairy producers and the integrity of the milk recording organization.

Sources of carryover contamination within recording devices

Collection of milk samples from participating dairy herds relies on the use of ICAR-approved recording and sampling devices. The age, type, and ownership of these devices vary among recording organization and represents significant investment by either the recording organization or the dairy producer. As it is unlikely that these devices may be replaced with new equipment, the goal should be the proper installation and operation of the existing devices. It is important to note that these devices were tested for accuracy with respect to the milk yield estimation and the ability to provide a representative milk sample across varying milk flow rates. There are no standards for acceptable carryover limits and no expectation of performance from manufacturers related to carryover as part of the ICAR approval test. There is little documentation related to the potential for carryover contamination from cow-to-cow within a specific recording device, nor can we assume that all recording and sampling devices will behave in a similar fashion. It is in the best interest of milk recording organizations to develop and implement best practices for the use of recording devices in both routine milk recording programs and for use in health or diagnostic screening programs using milk samples.

Table 2. Possible categories of recording devices for evaluation of potential for carryover.

Recording Device Class	Sampling Method
Monthly Meters (usually owned by milk recording organization)	
Flask	Manual sampling via alternating flasks
Valve Meters	Manual mixing and sampling through valve
Valve Meters with Sampler	Direct automatic sampling with/without mixing prior
Daily Meters (usually installed on the dairy)	
Weigh Jars	Total milk collection followed by mixing and subsampling
Fill and Dump Meters	Incremental (cycle) yield measurement with proportionate sampling via manufacturer's device
Continuous Flow Meters	Yield measurement by sensor, proportionate subsampling
Automatic Milking Systems	Direct sampling using external shuttle

When evaluating the suitability of a recording device and sampler for collection of these samples, there are two areas for consideration – a) the design of the meter and sampler, and b) the installation and maintenance of the meter and sampler. While both are important to the effectiveness of the milk recording system, it is important to recognize the distinction between the two focus areas. As suggested in Table 2, recording devices could be grouped for evaluation of carryover potential based on their design and method of sampling. This paper does not assign a carryover level or range to each grouping of recording devices, rather suggests that commonalities exists in the design that may predict similar behavior within a

group of devices. For example, a monthly recording device that alternates flasks for sampling offers more control over the mixing and complete emptying of the flask contents compared to other device types. In addition to the ability to completely empty the contents (remove visible residues) from the previous cow from flask or sampler, the subsampler size, potential for hidden residues in valves, hoses, tubes, and samplers, and the complexity of automatic milking system settings all affect the net carryover potential of a recording device.

For example, a device and sampler may have 3 mL of milk from the previous cow present (either visible or hidden) in the sampler tube or hose. If that same 3 mL of milk is transferred to a 25-30 mL sample vial directly, due to the meter's design, a potential of 10-12% carryover could exist. However, if that same 3 mL of milk is collected using a larger sampler (i.e. 80 mL) and then subsampled to the 25-30 mL vial, the potential carryover may be reduced to 3.75%.

Both of these carryover levels have the potential for false positives given the demonstrated sensitivity of current health screening tests; however, a two-thirds reduction in carryover reduces the likelihood carryover becomes problematic. However, with the reduction in potential carryover comes with the cost of additional labor to perform the subsampling process. Using the same logic, systems that collect a large subsample (200-500 mL) offer greater potential to reduce carryover contamination simply by dilution inherent in sampler design provided installation and operation are within specifications. In addition to considerations on residue from the previous cow and subsample size, the complexity of automatic milking systems offer additional challenges with respect to the potential of hidden residues but also to the reliance on automation and system programming. While the potential carryover behavior of a group of devices may be predicted based on operational principles, each device and sampler may have unique considerations, even more so with respect to specific automatic milking systems. Therefore, it is important to evaluate each system with diligence and respect to its proprietary design in determining suitability to provide samples for health screening tests.

There is currently a need to quantify the extent and location of both visible and hidden residues within milk recording and sampling systems to evaluate the carryover potential and develop guidelines and standards. However, one cannot overlook another tangible component of any milk recording device and sampler operation – the installation and maintenance of the system. This concept holds true for traditional milk yield measurement and component analysis, as well as health screening using ELISA and PCR testing. Devices owned and maintained by the recording organization traditionally offer more controls over routine maintenance and operation when compared to devices specifically installed on the dairy. As important as design, required maintenance and inspection ensure that the device and sampler are performing within manufacturer's specifications and providing an accurate and representative subsample to the laboratory for analysis with minimal carryover. If not already in place, milk recording organizations should require periodic inspection and preventative maintenance of all recording devices owned by the organization and by dairy producers to ensure equipment is suitable for herd recording.

It is also the responsibility of the milk recording organization to ensure that training programs for employee technicians are in place to minimize the potential carryover during the collection of milk samples. Regardless of the extent of the technology associated with the

recording device, there is a human component associated with the collection, identification, and handling of milk samples. In addition to technician training, development and dissemination of resources to aid dairy producers who collect their own samples should be a part of offering diagnostic screening tools.

Practical application by the recording organization

As each new diagnostic test is brought to the marketplace, there is an immediate desire to market these health screening tools to dairy producers. The use of milk samples collected during the herd recording day primarily for analysis of endogenous components offers the dairy producer a convenient and cost effective tool for individual animal health screening. In addition supplemental tests can provide an additional source of revenue for the recording organization. However, as milk recording organizations expand into these markets, assurances that appropriate samples are being provided will be critical. This will require an evaluation of the recording devices used for sample collection to ensure a representative sample - which can provide an uncontaminated substrate for the test with far greater sensitively than infrared analysis for milk fat or protein - is consistently available. It is important to note that each ICAR-approved recording device has met the existing requirements and the device manufacturer offers an accurate meter and sampling device for its original intended use on the dairy. It is the recording organization's expansion of the analyses performed on the milk sample that brings cause to revisit the uniqueness of each sample collected.

There is certainly no expectation that a recording device would operate with zero carryover potential. However, with the measurement of the carryover potential associated with differing recording devices, it will be possible to ascertain the suitability of using a specific recording device for the collection of a milk sample for a specific type of testing for exogenous components. Further, the quantification of carryover potential may identify the source(s) of carryover associated with specific devices or types of devices, providing relevant data for development and implementation of best practices in the installation and maintenance of the recording devices as well as sampling protocols. Assuming the application of these best practices by the recording organization, a decision tree may be developed to determine the eligibility of the milk sample for health screening analysis by ELISA and/or PCR analysis, providing greater assurance of accurate results and an improved reputation of the milk recording organization and laboratory conducting these tests.