

Proposed Method for Using Milk Line Sampling in Raw Milk Bacteriological Quality Testing

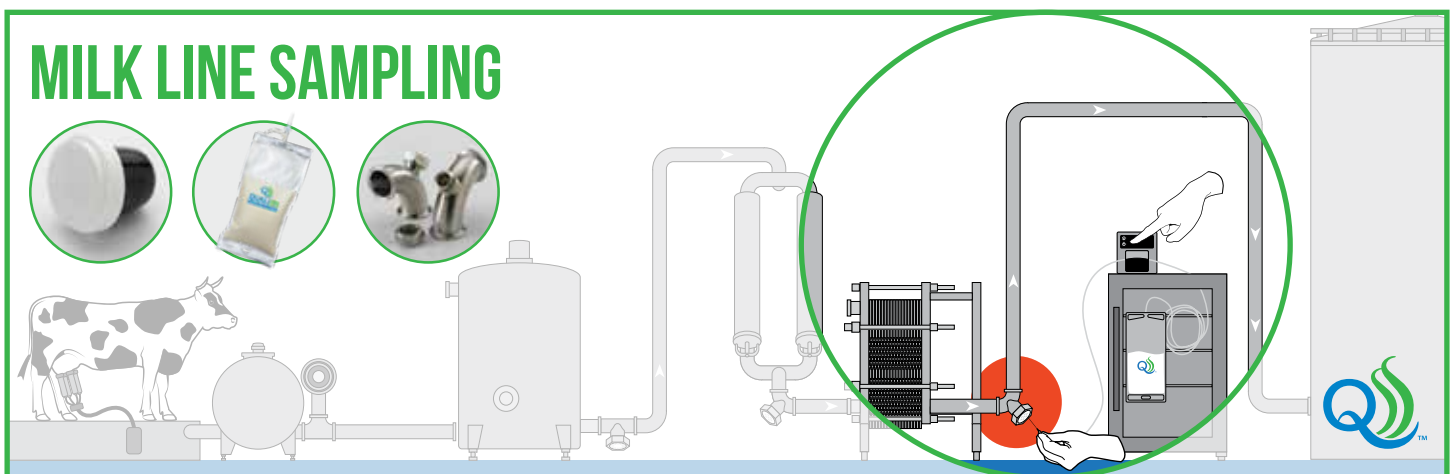
Introduction

Sampling and culturing bulk tank milk (BTM) to help establish the bacterial health of a dairy herd has come into practice since the 1970s.¹ The National Mastitis Council (NMC) cautions, however, that the method has "...not been adequately evaluated under controlled scientific conditions" and that results should be interpreted by practitioners that fully understand the limitations of the method.²

According to the NMC, the BTM method is limited to producing two types of data that include determining the presence or absence of a bacterial group and identification of predominant bacterial groups in the BTM. The line sampling method using the QualiTru TruStream Elbow and TruStream7 Septum in milk sample collection may address many shortcomings of the BTM method and could potentially be a useful surveillance tool for maintaining herd health and milk quality. In 2002, Godden, et al. reported there are four potential benefits to line sampling as part of a routine cow health and milk quality monitoring program³:

1. A line sampling program allows producers to monitor different groups of cows within the herd, so called "pen" or "string" sampling where a routing testing program could be implemented relatively inexpensively. Notably, more frequent monitoring should have the effect of increasing test sensitivity, or the ability to detect changes in a group's performance.
2. A line sample may provide greater test sensitivity for detection of pathogens because it allows sampling relatively small groups of cows at a time rather than the whole herd or large groups. Sampling smaller groups means there is less dilution effect in the sample, enhancing the probability of detecting pathogens.
3. Milk line sampling may be used to narrow the search for cows that are infected with contagious mastitis pathogens stating, "If the culture of milk...from individual groups could be used to narrow the search to one or two positive groups, this would have potential to save the producer considerable time, labor, and expense."
4. A very useful prospect for using milk line sampling is to use counts of environmental bacteria, such as coliforms or *Streptococcus non-agalactiae* to monitor changes in environmental sanitation and milking management.

Importantly, a milk line sampling method has not been specifically validated through controlled scientific studies. This Proposed Method represents a compilation of methods that have been used successfully in various scientific studies reported in scientific or lay literature.





Sample Collection

(adapted from Godden, et al.³)

1. Sampling should occur over the continuous milking of the string and should end when milking of the string has concluded.
2. Install the QualiTru TruStream Elbow in the milk line downstream of the receiver jar and plate cooler if present. Placing additional elbows in the line and collecting repetitive samples may improve test sensitivity.
3. Position the elbow such that the sampling port is on the bottom or side of the line, if possible.
4. Insert the new septum into the TruStream stainless steel port. Remember to only touch the outside of the rim of the septum. Place the nut over the septum, hand tighten the nut and then tighten 1/8 turn with a wrench. Swab the outer surface of the septum with sanitizer. The nut may need to be re-tightened periodically due to vibration and cleaning cycles.
5. Insert an 18-gauge needle through the septum channel diaphragm with the bevel of the needle toward the outside of the septum. In the event the septum was positioned on the top of the line contact a QualiTru representative to advise on the correct needle method.
6. Connect the sample collection line to a sterile TruMotion Collection Bag. Place the bag in an insulated cooler surrounded by ice or ice packs.
7. Connect the sample collection line to a QualiTru Portable Peristaltic Pump or Watson Marlow Peristaltic Pump. Set the pump flow regulation to collect 10 to 15 milliliters (ml) each time the pump operates. QualiTru recommends that a total sample volume of 1500 ml or greater should be collected over the course of the milking to help ensure that the sample is representative. Download the [Watson Marlow Peristaltic Pump Usage Guide](#) from the resources page of our website to review how to set the correct setting.
8. Upon completion of the milking process, the sample collection container(s) should be removed to the laboratory and held in a refrigerator until tested. Bacteriological testing should occur as soon as possible following the milking.

Bacteriological Testing

(adapted from NMC²; Farnsworth¹; and Karmaoui and Farnsworth⁴)

1. Remove samples from refrigerator and mix the sample thoroughly. Download the [Proper Agitation of a Sterile Collection Bag](#) guide from the Standard Operating Procedures (SOPs) page of our website for more information.
2. Because many organisms of interest may occur in low concentration, plating the largest volume of milk possible increases the probability of finding them. To start, samples are plated by streaking a 200 microliter (µl) (0.2 ml) aliquot of sample evenly across the entire surface of an agar plate using an L-shaped dally rod. A 200 µl test volume provides a 1:5 dilution of the milk sample. Further dilutions of 1:50 and 1:500 can then be made easily. This range of dilutions should be adequate to give satisfactory results from all samples, but larger aliquots may be tested using a pour plate technique, if needed.
3. Each sample should be plated in duplicate on trypticase soy agar containing 5% bovine blood and 0.1% esculin (TBA), MacConkey agar (MAC), TKT agar, and mannitol salt agar (MSA) or Staphylococcal Medium 110 (SM).
4. Incubate at 37°C for 48 hours.
5. Counts from duplicate plates are averaged and recorded as colony forming units (CFU) per milliliter for total bacterial growth (total growth on TBA); gram-negative bacteria growth (MAC); coliforms (lactose-positive colonies on MAC); streptococci (TKT); and staphylococci (MSA or SM).
6. Suspect *Staphylococcus aureus* colonies are confirmed by the tube coagulase test or by an anti-protein A and clumping factor latex agglutination test. Suspect *Streptococcus agalactiae* colonies are confirmed by the CAMP reaction or Lancefield group B latex agglutination reaction.

Contact us 651.501.2337 to learn more about quality assessment methods or visit our website at QualiTru.com!

References:

1. Farnsworth RJ. 1993. Microbiological examination of bulk tank milk. *Vet Clin North Am: Food Animal Prac.* 9(3):469-474.
2. National Mastitis Council. Using Bulk Tank Milk Cultures in a Dairy Practice. National Mastitis Council Protocols, Guidelines and Procedures. <https://www.nmconline.org/nmc-protocols-guidelines-and-procedures/>
3. Godden S, R Bey, R Farnsworth, J Reneau, M LaValle. 2002. Field Validation of a Milk Line Sampling Device for Monitoring Milk Quality and Udder Health. *J Dairy Sci.* 85:1468-1475.
4. Karmaoui P and RJ Farnsworth. 1993. Practical Laboratory Techniques for Identifying Mastitis-Causing Organisms. University of Minnesota, College of Veterinary Medicine, Veterinary Continuing Education.