



**Wisconsin Veterinary
Diagnostic Laboratory**
UNIVERSITY OF WISCONSIN-MADISON

Milk Sample Collection

Milk samples may be collected individually from each affected quarter (quarter milk samples) or combined from multiple quarters of a cow into one sample tube (composite milk samples). Composite milk samples are not recommended however, as cultures usually reveal growth of numerous different bacterial species, making it difficult, if not impossible, to determine which pathogens are causing mastitis and which are environmental contaminants. Isolation of contagious organisms, such as *Staphylococcus aureus*, *Streptococcus agalactiae*, or *Mycoplasma sp.*, are indicators of true infections of the udder. Environmental organisms, such as *Streptococcus spp.*, coliforms, *Staphylococcus spp.*, (coagulase negative *Staph.*), *Pseudomonas sp.*, *Corynebacterium sp.*, yeast, and fungi, may be contaminants or true infections. Unless only screening for contagious pathogens, composite milk samples should be avoided.

Milk samples may become contaminated with bacteria from the hands of the sample collector, the environment, and the teat, skin or teat canal of the cow. It is important that proper sample collection techniques are used in order to avoid contamination of the milk sample.

Follow these steps to aseptically collect milk samples for culture:

1. Using a permanent marker, label a new sample tube with the date, cow ID, and the quarter that the milk will be collected from (RF for right front, LF for left front, RR for right rear, LR for left rear). Keep the sample tubes closed until the sample will be collected to prevent contamination of your milk collection tubes.
2. Wash your hands and put on new disposable gloves.
3. Make sure that the udder and teats are clean and dry before collection. Pre-dip the teats with an effective germicidal teat dip and leave the dip on for 30 seconds.
4. Wipe each teat dry with a single-use paper or cloth towel, making sure there is no teat dip left behind on the teat. Residual teat dip left on the teat will kill the bacteria in your milk sample.
5. Discard 3 to 4 streams of milk to minimize risk of contamination of the sample with bacteria in the teat canal.

6. Scrub teat ends with a cotton ball or gauze pad soaked in alcohol. Scrub until the cotton ball or gauze pad comes away clean (no dirt/debris can be seen). If sampling more than one quarter of the same cow, scrub far teats before scrubbing near teats. Use a new cotton ball or gauze pad for each teat. Teats should not be dripping with alcohol, as the alcohol will kill the bacteria in your milk sample.
7. Open the sample tube immediately before the sample is taken. Do not let your hands or the teat end come into contact with the inside of the tube, including the lid. Collect milk until the sample tube is $\frac{1}{3}$ to $\frac{1}{2}$ full, holding the tube at an angle to prevent loose dirt or hair from falling into it. Immediately close the tube once filled, as indicated.
8. Immediately put the sample tube in the refrigerator or on ice. Samples that will not be plated within 24 hours should be frozen. It is best to freeze samples before shipping to the lab.

Additional Recommendations:

- Plastic tubes with snap-on lids work best for collecting milk samples. Whirl pack and Ziplock bags should NOT be used as they are easily contaminated during sample collection and also often leak during transportation.
- To avoid contamination, handle sample tubes properly to ensure sterility at all times. Make sure nothing but the sample milk comes into contact with the inside of the tubes.
- Check that sample tubes are no more than $\frac{1}{2}$ full and that lids are completely closed to avoid leakage or bursting upon freezing (milk will expand when frozen).
- Collect samples directly from teats. Bucket or milk meter samples carry over bacteria from previous cows.
- The best time to sample is at milking time before the cow is milked. If the sample is not collected at milking time, it should be taken at least 4 hours after the last milking.
- Label the sample tube with a permanent marker before sample collection, as milk fat will cause the ink to smear.
- For composite milk samples, it is ideal to collect the same volume of milk from each quarter.
- Minimize contamination by collecting samples in a clean area, such as the parlor. Avoid areas with massive air movement where bedding and dust can cause major contamination problems.



- Make sure samples are cold or frozen until they are delivered to the lab to avoid excessive growth of bacteria within the sample, which can lead to misleading results.

Bulk Tank Milk Samples

Bulk tank milk cultures are a great way to monitor milk quality. They can determine the presence or absence of bacteria and identify predominant bacterial groups within bulk tank milk.

Follow these guidelines when collecting bulk tank milk samples:

- Agitate the milk in the bulk tank for 5 minutes before sampling.
- Always collect the sample from the top of the bulk tank and never from the outlet, as milk collected from the outlet is often contaminated.
- Use a clean sanitized dipper or sterile syringe to collect the sample.
- Fill the sample tube ½ full, as milk expands when frozen.
- Immediately place samples on ice or in the refrigerator. Freeze samples that will not be plated within 24 hours.
- Collect bulk tank milk samples 3-5 days in a row. This allows for greater accuracy than single day sampling for contagious pathogens.
- DO NOT FREEZE samples for Prototheca culture. If testing for Prototheca, milk samples should be refrigerated and not frozen. Bulk samples may be collected in duplicate and one sample save under refrigeration until submission for prototheca culture.

Reference:

Ruegg, P., Godden, S., Lago, A., Bey, R., Leslie, K., *On-Farm Culturing for Better Milk Quality*, <http://wdmc.org/2009/On%20Farm%20Culturing%20for%20Better%20Milk%20Quality.pdf>. Accessed on 2/16/2022.