

Best practice sample submission

Milk samples

Sample Selection - Composite vs. Quarter Samples

The first step of milk sample submission is selecting the right test, as the test will dictate the type of sample to collect. Composite (all four quarters collected into one tube) versus individual quarter samples have different uses.

Submit Composite or Quarter Samples for Screening Cultures:

Composite samples are used for cow-level screening, where the decision that will be made based on the culture result is at the cow level (such as culling or treating all four quarters). The benefit of composite sampling is reduced cost for testing, however a large percentage of composite samples will grow multiple organisms. When three or more organisms are isolated from a milk sample it is considered contaminated, and it is difficult to tell which, if any of the organisms originated in the udder (causing infection) and which are environmental organisms that contaminated the sample during collection. Contagious mastitis pathogens are more likely to have originated in an infected udder. Screening cultures detect and report the following contagious pathogens, even in samples that are contaminated:

- Mycoplasma
- Prototheca
- Staphylococcus aureus and Mycoplasma
- Staphylococcus aureus and Streptococcus agalactiae
- Staphylococcus aureus
- Streptococcus agalactiae

The presence of excessive contamination may make it impossible to find the pathogen of interest, in which case the lab will report "Inconclusive due to overgrowth." Therefore, it is still important to collect composite samples aseptically (see sample collection protocol below).

Note that the ability to detect an infection in a single quarter will be reduced when using composite samples due to dilution of the infected milk with milk from healthy quarters. For that reason, you may choose to submit quarter samples instead of composite samples for screening cultures in some cases where greater diagnostic accuracy is desired.

Submit Quarter Samples for Full Mastitis Culture:

Aseptically collected quarter milk samples are preferred for Full Mastitis Cultures. A quarter is seldom infected with more than one organism at the same time. The Lab for Udder Health will report up to two organisms isolated from samples submitted for Full Mastitis Culture; if three or more organisms are found, the results will be reported as “Contaminated.”

Sample collection - Quarter or Composite Milk Samples

Proper sample collection technique is very important to avoid sample contamination and erroneous culture results. Please review the following guide before collecting samples to help minimize sample contamination

- Collect the following supplies: clean, disposable gloves, waterproof marking pen, milk sample tubes, cotton balls or pads soaked in 70% alcohol, cooler with ice or freezer packs, germicidal teat dip, paper or cloth towels.



- Wash your hands and then put on new disposable gloves. Label the sample tube using a waterproof marking pen. Clearly record the date, the cow ID and the quarter from which the sample will be taken. RF = right front, LF = left front, RR = right rear, LR = left rear.



- Brush off any loose manure, dirt or bedding particles from the udder and teats. Pre-dip with an effective germicidal teat dip, leaving the dip on for 30 seconds. (If the udder and teats are extremely dirty, thoroughly wash and dry the udder and teats before pre-dipping.)



- Wipe each teat dry with a single-use paper or cloth towel, paying particular attention to the teat end. Be sure there is no teat dip remaining on the teat, as it will kill bacteria in your milk sample.



- Discard 3 to 4 streams of milk on the floor to minimize chances of contaminating the sample with bacteria in the teat canal.



- Scrub teat ends using a cotton ball or gauze pad soaked in alcohol. Scrub until the ball or pad comes away clean, using as many as necessary. If sampling more than one quarter of the same cow, scrub far teats first, followed by near teats to avoid re-contaminating teats you have already scrubbed. Use a new swab for each teat. Teats should not be dripping with alcohol, as this will also kill any bacteria in your milk sample. Do not touch the teat ends again after this step.



- Open the sample vial immediately before the sample is taken, not before. Do not touch the inside of the vial or cap or let the teat end touch the vial. Hold the vial at an angle to keep loose dirt or hair from falling into it. Direct streams of milk into the vial without touching the teat end. Sample as quickly as possible, starting with near teats first, followed by far teats. Fill the vial 1/3 to 1/2 full. Immediately close the sample vial so that it is airtight. Collect milk from each quarter into a separate vial (quarter samples).
- Immediately place the sample vial on ice or in the refrigerator. Keep samples on ice or in the refrigerator until plated and freeze samples that will not be plated within 24 hours. It is best to freeze samples before shipping to the lab.

Additional Tips

- Plastic flip-top tubes work best for collecting milk samples. Whirl pack bags should not be used because they frequently become contaminated during sampling and leak during shipment.
- Sample tubes should be handled properly to ensure sterility at all times. Do not put caps into pockets, touch the tops or touch the inside of the collection tubes. Avoid getting particles of dust, dirt, or manure into/on the sample tube.

- Ensure that tubes are filled no more than half full and lids are completely closed. Over-filling tubes makes it more likely that tubes will become contaminated or burst when frozen.
- Samples should be taken directly from teats. Bucket or milk meter samples carry over bacteria from previous cows.
- If collecting composite samples, remember to prep the two teats farthest from you first, then the two teats closest to you, and sample in the reverse order to prevent contamination. Or, you may want to prep and sample the far teats, then the near teats. If any teat is touched between cleaning and sampling, re-prep that teat before collecting the sample.
- It is best to sample at milking time (before milking the cow). If the sample is not taken at milking time, it should be taken at least 4 hours after the last milking.
- If possible, label the top of the collection tube with the proper cow and quarter identification number prior to sample collection (milk fat will cause markings to smear). Use a waterproof permanent marker.
- If collecting milk from all quarters into the same tube (composite sample), try to take the same amount of milk from each quarter.
- Sampling in a clean location (such as a parlor) reduces the likelihood of contaminants falling into the sample. Open barn doors or tunnel ventilation can cause massive air movement, resulting in major contamination problems from bedding and dust.
- Be aware of manure on your clothing or hands and wash or change before collecting samples if needed.
- Samples should be kept cold or frozen until delivery to the Minnesota Veterinary Diagnostic Laboratory. Bacteria in samples that are not cooled or frozen may grow excessively, resulting in misleading results. If you will be storing samples for some time before shipping, follow these guidelines:
 - All samples that will not be shipped immediately after collection via same day or overnight shipping to the Lab for Udder Health should be frozen before shipping.
 - Samples submitted for aerobic culture (Full Mastitis or Screening Culture other than Mycoplasma) can be frozen for up to 1 month before shipping.
 - Samples submitted for Mycoplasma culture should be frozen for no more than 2 weeks, as longer freezing time may decrease recovery of Mycoplasma.

Sample collection - Bulk Milk Samples

Please follow these steps carefully to avoid contamination:

- Collect samples 3-5 days in a row. Five day sampling procedures achieve more accuracy than single day sampling for contagious pathogens such as Staph aureus.
 - Multiple samples for one bulk tank test are pooled, unless otherwise noted on the submission form. Larger herds may choose to test day or tank samples individually, or string sample, to increase sensitivity.
- Agitate the tank well before sampling. Use a clean dipper or sterile syringe to draw the sample from the top of the tank. If using a syringe, collect approximately 5-10cc. Empty the syringe immediately into a clean milk collection tube. Fill the tube one-half full (Remember, milk expands when frozen).
- Please do not send milk samples in a syringe as the plunger frequently comes out during transit and introduces contamination.
- Place immediately in the coldest part of the freezer. Do not delay! Extensive delays will allow bacteria to grow giving erroneous results

IMPORTANT: Do not collect samples from the outlet valve unless absolutely necessary because samples collected in this manner often will be contaminated. If you must collect from the outlet valve, allow 1-2 gallons of milk to run before collecting the sample (you can catch the excess milk cleanly and return it to the tank).

Submission form

- Complete the Laboratory for Udder Health submission [form](#). Please contact the lab if you have any questions about completing the submission form or need assistance with test selection.
- Samples from different sites may be included in the same shipping box, but there should be a separate submission form included for each site/location. This ensures that the appropriate tests are performed and results are available to the proper parties.
- One submission form can be used for multiple test requests as long as the samples are clearly labeled. For example, you may submit quarter milk samples for Full Mastitis Culture, along with composite samples for Screening Culture. Please make sure it is very clear which tests apply to which set of samples (perhaps bag samples separately and label the bag with what test is requested).
- Seal submission forms in a separate bag from the samples being submitted.

Packaging & shipping

- Tubes should be placed in a sealed bag to keep the sample(s) contained in case there is any leakage.
- Samples should be frozen before shipping to ensure that bacterial overgrowth does not occur during shipping.
- Ship samples in an insulated shipping container with freezer packs.
 - Liquid samples submitted in whirl pacs or other bags will be subject to an additional handling fee.
- Fill empty space in the container with packing material to prevent tubes and ice packs from moving around inside the box during shipping.
- Styrofoam boxes specially designed for milk sample shipment are available from the Laboratory for Udder Health. These boxes contain cold packs and 15 sterile tubes for shipping bulk tanks or individual cows. [Order form and information](#)