



Taking Sterile Milk Samples

Background:

Part of mastitis control programs include microbiological analysis of milk from cows suspected of having mastitis. Culturing milk samples allows the identification of the bacteria that are causing the mastitis and the application of preventive management programs. Strict aseptic procedures must be used when collecting milk samples to avoid contamination with bacteria present on the skin of the cow, hands of the sampler and barn environment.

Equipment:

- Sterile single use disposable plastic vials with tight fitting caps
 - Vials should be at least 15 ml. Capacity.
- Nitrile or latex gloves should be worn to reduce contamination of samples with bacteria present on the samplers' hands.
- Alcohol soaked cotton, gauze or baby wipes are needed for adequate teat sanitation.
- Vials should be labeled with permanent markers to identify the cow and quarter being sampled.
- If multiple samples will be collected, racks should be used for convenient handling.

Individual Quarter Milk Sample

Udders and teats should be clean and dry prior to individual quarter sample collection.

- A strip cup can be used to examine a cow suspected with Clinical Mastitis.
- Forestripping 3 streams of milk from the teat to be sampled removes contaminated milk from the teat canal. The use of this practice will reduce the likelihood that unusable contaminated samples will be obtained.
- Teat sanitation can be accomplished through the use of Predipping with 0.5% iodine. The disinfectant must remain on the teats for *20 to 30 seconds* prior to removal.
- It is important to *thoroughly dry the teat* with a single use cloth or paper towel.
- Special attention should be paid to the teat end to achieve adequate sanitation.
- 70% ethyl or isopropyl alcohol must be used to fully sanitize the teat end prior to obtaining the milk sample. The scrubbing of the teat end should be vigorous to fully sanitize the teat.
- Alcohol is an ideal antiseptic because it evaporates quickly and will not contaminate the milk sample. If multiple teats are sampled a separate swab must be used for each sample.
- Sanitation is not complete until the surface of the swab remains clean after it is used.

- The cap should be removed from the sample vial without touching the inside and it should be held so that the inner surface faces down. This will prevent sample contamination.
- The vial should be held at an angle so that debris does not fall into it.
- Milk from the teat to be samples can be directed at an angle into the sampling vial. A sample size of 3-5 ml is usually adequate.
- The cap should be immediately replaced after the sample is obtained.

Composite Milk Samples

Individual quarter samples are the most sensitive way to determine the type of mastitis pathogen that is present, but sometimes a “composite sample” is collected. The term composite milk sample refers to the collection of milk samples from all 4 quarters into a single sample vial. This type of sample is often used in herd screening programs for contagious mastitis pathogens such as *Strep agalactia* or *Mycoplasma bovis*. Composite samples are used to reduce the cost of sampling but generally result in some level of false negative results.

- Predipping is the first step in obtaining a composite milk sample.
- The process of teat preparation for obtaining composite milk samples is identical to that of obtaining individual quarter samples but the order of teat preparation is critical. To reduce cross teat contamination, the far teats should be sanitized before the near teats. Individual alcohol swabs should be used to sanitize each individual teat.
- After teats are prepared, an equal volume of milk should be obtained from each quarter into the same vial. The order of sampling is near teats before the far teats.
- Immediately after culturing, the milk samples should be placed on ice or in a refrigerator. The milk should be cultured within 24 hours of obtaining the sample.
- If samples cannot be cultured within 24 hours, they must be stored in a freezer as soon as possible. Isolation of staph and strep may be improved by freezing. The number of samples positive for *E. coli* may decline after freezing.

SUMMARY:

The correct steps for taking sterile milk cultures are:

- Use Proper Equipment
- Clean, Dry Teats and Udders
- Forestrip Teats to be Sampled
- Predip Teats
- Dry Teats
- Sanitize Teat Ends
- Take Sample
- Refrigerate or Freeze Sample