Bovine milk samples collection

Introduction

Milk samples can be used to measure the fat and protein content very easily. In order to investigate more components in the Milk it is essential to store and treat the milk under the wright conditions. We will collect from every lactating cow 2 milk samples.
In developing individual farm **mastitis control and treatment** strategies, it is often necessary to characterize the types of bacteria that are present on your farm. To answer this question, a microbiological analysis, or milk culture, must be performed on milk samples collected from cows showing clinical or subclinical signs of mastitis. Results of the milk cultures will help identify which bacteria are causing the mastitis. In turn, this information can be used to alter mastitis control, prevention, and treatment options to fit your herd's conditions.
During an investigation of a herd dealing with **high somatic cell counts** or a high incidence of clinical mastitis, milk culture results provide essential evidence for solving the problem. When managing a contagious mastitis problem (*Staphylococcus aureus* or *Streptococcus agalactiae*), milk cultures are even more important to help make individual cow treatment and culling decisions. Extra care and precaution are necessary during the collection process, using strict, clean, aseptic (without germs and bacteria) procedures to be sure that the bacteria originated from milk from the udder and not from the teat end or hair, the sampler's hands, or the barn environment. If the samples are not collected, handled, and transported correctly, the bacteriological results will be of no diagnostic value.

Necessary Supplies:

- Sterile single-use disposable 50ml plastic cellstar tubes with screw cap. (greiner Bio-one art nr. 227261) or similar
  Prepare per animal:
  1x 50ml tubes with 5ml 0.3% NaNA (sodium azide) (label with **animal ID** and **NaNA**
  1x 50ml tube with no additives labelled with **animal ID**
- Nitrile or latex gloves should be worn by the person collecting the samples.
- 70% ethyl alcohol
- -0.3% Sodium azide (NaNA, Sigma Adrich S2002-500) careful solution is extremely toxic
- A marker pen waterproof
- Alcohol-soaked cotton balls or gauze pads should be used to sanitize the teat ends or Baby wipes can be used to clean the tits
- a rack may be needed to handle sample vials.
- A small cooler (coolbox) or ice chest may also prove useful to immediately chill the samples after collection.
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Common Problems:

2. Udders and teats not cleaned properly.
3. Samples taken from cows on antibiotic therapy.
4. Identification numbers on containers are not legible or have been wiped off.
5. Incorrect number orientation (e.g., 18 or 81).

6. Forms not adequately filled out.
7. Samples not transported to the laboratory within 24 hours.
8. Samples not chilled during transport.
9. Sample containers broken or leaking during transport.

Milk Sample Collection:
Milk samples must be collected before milking or at least 1hr after milking the cow when the cows are milked. When suckler cows are sampled no time restrictions are needed . When individual quarters show clinical signs of mastitis, please record.

To minimize contamination and maximize the chances of receiving useful information from the milk culturing process, adhere to the **guidelines as much as possible** for the semi-aseptic collection of clean milk samples below.

The Collection of Clean Milk Samples (protocol is also illustrated in Figure 1):

1. Wear gloves.
2. Remove (forestrip) three or four streams of milk from the quarters being sampled to minimize chances of sample contamination from **bacteria** in the teat end.
3. Brush any dirt, debris, or bedding particles from the udder and teats. use baby sanitary wipes to clean the tits.
4. Dry each tit thoroughly by using a single, dry paper or cloth towel per cow with particular emphasis on the teat end.
5. Double check to ensure that the teats and udder are clean and dry.
6. Collect milk in a 0.5 liter jug. Hold the collection jug at a 45° angle to keep debris (hair, manure, dirt) from accidentally falling into the jug. Turn the teat toward the jug, striving for direct streams of milk into the jug. The teat should never touch the jug. Sample as rapidly as possible, starting with the teats on the near side of the udder followed by the teats on the far side of the udder.
7. Combine the milk from the different teats
8. Collect 100ml of milk
9. Label the sample vials using a waterproof marker that will not come off during transport to the laboratory.
10. Fill the two 50ml tubes and mix by turning upside down 10 times
11. Immediately place collection vial on ice and keep refrigerated or on ice until delivered to the lab. Best results are obtained if samples are chilled or placed on ice during transport to the laboratory.

Laboratory:

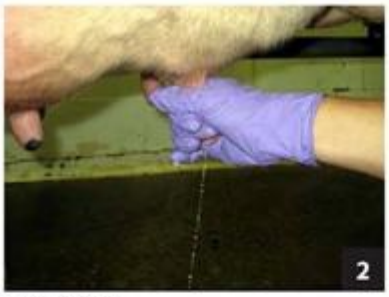
- Freeze milk with sodium azide at -80°C

- Add 50ul 0,5M EDTA to 50ml fresh milk without adatives and gently mix
- Centrifuge: 540g; 10 minutes; 4 degrees
- Scoop the fat (which forms a top layer) with a spoon (spatula or disposable coffee stirring rod (single use, autoclaved) and transfer to 1,5 ml eppendorf tube. (normal amounts are between 0,5-1,5 ml) add **animal id** and **milk fat** on the tube.
    - ✓ Pour supernatant carefully in a new 50ml tube. Make 4 1.5 ml tubes with 1ml milk serum and 2 2ml tubes with 1.5 ml milk serum (labelling see below).

- ✓ 1ml in 1,5ml tube > major proteins (tube labelled with **animal id** and **major protein**)
- ✓ 1ml in 1,5ml tube > major proteins (tube labelled with **animal id** and **major protein**)
- ✓ 1ml in 1,5ml tube > minor proteins (tube labelled with **animal id** and **minor protein**)
- ✓ 1ml in 1,5ml tube > salt (tube labelled with **animal id** and **salt**)
- ✓ 1,5ml in 2ml tube > serum (tube labelled with **animal id** and **milk serum**)
- ✓ 1,5ml in 2ml tube > serum (tube labelled with **animal id** and **milk serum**)

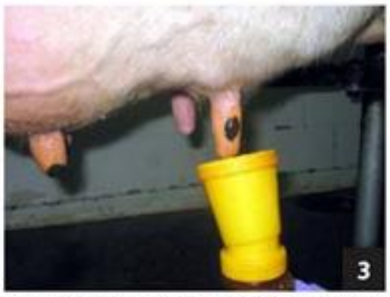
- Wash pelleted cells with 10 ml PBS-EDTA (add 10ul 0,5M EDTA to 10ml 1xPBS pH7,2)
- Centrifuge: 540g; 10 minutes; 4 degrees
- Discard supernatant
- Repeat this washing step to remove fat and proteins completely
- Resuspend pellet in 500ul qiazol (liquid reagent: Qiagen Sciences # 56304569). Transfer to a new 1.5 ml tube (labelled with **animal id** and **milk RNA**).
- Store all sample tubes (1x fat, 6x milk serum, 1x cells in qiazol) at -80 degrees (Can be stored for longer time at -80)

Collecting Clean Milk Samples

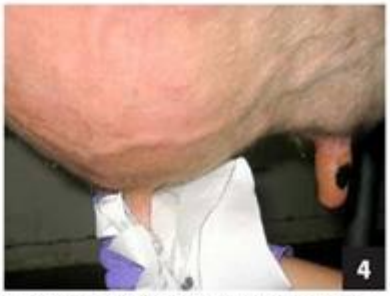


1. Wear gloves.

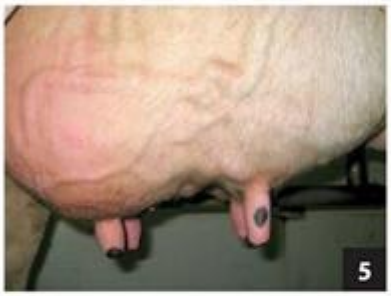
2. Remove (forestrip) 3 or 4 streams of milk from the quarter being sampled to minimize chances of sample contamination from bacteria in the teat end.



3. Brush any dirt, debris, or bedding particles from the udder and teats. Predip with an effective teat dip (for example, 0.5% iodine or 4% hypochlorite) leaving the predip on the teat for at least 20 to 30 seconds before removal.



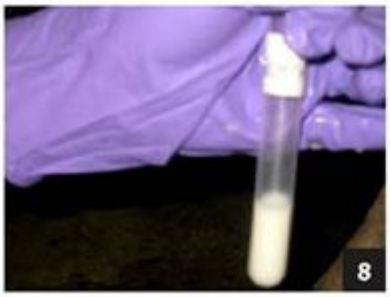
4. Dry each teat thoroughly and remove the predip using a single, dry paper or cloth towel per cow with particular emphasis on the teat end.



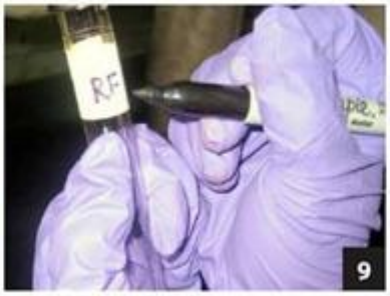
5. Double-check to ensure that the teats and udder are clean and dry.

6. For 15 to 20 seconds, carefully and vigorously scrub the teat end and orifice with a cotton or cloth gauze pad moistened (but not dripping wet) with 70 to 80% ethyl or isopropyl alcohol. Use a separate swab for each teat being sampled, even within the same cow. Continue to clean the teat end until the swab is completely clean and white. In order to prevent recontamination of teat ends, clean the teats on the far side of the udder first and followed by the teats on the near side of the udder.

7. Open the collection vial immediately before the sample is taken. Do not let the teat end touch the container or let skin debris or dirt enter the container. Do not put the cap on the floor. Keep the cap upside down and do not touch the inside of the cap so that no debris contaminates the inside of the cap. Hold the collection vial at a 45° angle to keep debris (hair, manure, dirt) from accidently falling into the collection vial. Turn the teat toward the collection vial, striving for direct streams of milk into the vial. The teat should never touch the collection vial or cap. Sample as rapidly as possible, starting with the teats on the near side of the udder followed by the teats on the far side of the udder.



8. You only need to collect 3 to 5 ml of milk (a few streams). Do not fill the collection vial. Attempting to fill the collection vial increases the likelihood of contamination. In addition, if a full collection vial is frozen, it may burst. Immediately place cap on container and seal so it is air tight.



9. Label the sample vials using a waterproof marker that will not come off during transport to the laboratory. Be sure to identify both the cow and quarter from which the sample was obtained. Designate each quarter sampled as RF (right front), RR (right rear), LF (left front), or LR (left rear).

10. Immediately place collection vial on ice and keep refrigerated or on ice until delivered to the lab. Best results are obtained if samples are chilled or placed on ice during transport to the laboratory. When samples cannot be delivered to the laboratory within 24 hours, they should be frozen.

Figure 1. Collecting clean milk samples.

Additional Tips:

 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 ½ full and that lids are completely closed to avoid leakage or bursting upon freezing (milk expands 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, try to collect the same volume of milk from each quarter.
 Minimize contamination by collecting samples in a clean area as much as possible, 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, which can lead to misleading results.