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Collection and Preparation of Milk Samples for Microbiological Culturing

Jeffrey M. Bewley and Robert J. Harmon, Animal and Food Sciences; Stephan Locke, Livestock Disease Diagnostic Center

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 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.



Common Problems

1. Udders and teats not cleaned properly.
2. Samples taken from cows on antibiotic therapy.
3. Identification numbers on containers are not legible or have been wiped off.
4. Incorrect number orientation (e.g. 18 or 81).
5. Forms not adequately filled out.
6. Samples not transported to the laboratory within 24 hours.
7. Samples not chilled during transport.
8. Sample containers broken or leaking during transport.

Necessary Supplies

Sterile glass or single-use disposable plastic vials with tight-fitting push-on or screw caps should be used to collect milk samples. Plastic sample bags are **not** an acceptable collection device. To reduce potential contamination from the sampler's hands, nitrile or latex gloves should be worn by the person collecting the samples. Alcohol soaked cotton balls or gauze pads should be used to sanitize the teat ends. If a large

number of samples are being collected, a rack may be needed to handle sample vials. A small cooler or ice chest may also prove useful to immediately chill the samples after collection.

Milk Sample Collection

Milk samples must be collected before the cow is treated with antibiotics. Samples for culture should be collected immediately before milking. When individual quarters show clinical signs of mastitis or positive California Mastitis Test results, individual samples should be collected from the affected quarters. If the entire herd is being sampled, composite samples (all four quarters in one collection vial) will provide reasonable results.

To minimize contamination and maximize the chances of receiving useful information from the milk culturing process, adhere to the guidelines for the aseptic collection of clean milk samples on the next page.

Handling and Shipment

If more than 20 samples are to be submitted to the University of Kentucky Livestock Disease Diagnostic Center in Lexington, Kentucky, the Bacteriology section (859-253-0571) must be notified at least one week before they are submitted. If more than 10 samples are to be submitted, they should be received by the laboratory on Monday, Tuesday, or Wednesday for best diagnostic results. Sample culture requires two to seven days for completion. If more than 10 samples are submitted, the tube numbers corresponding to the cow numbers or names should be listed on a separate piece of paper. Culture results will auto-

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.



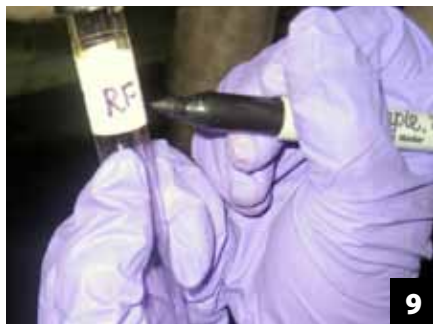
6. For 15 to 20 seconds, carefully and vigorously scrub the teat end and orifice with a cotton or cloth gauze pad moistened (but not dripping wet) with 70 to 80% ethyl or isopropyl alcohol. Use a separate swab for each teat being sampled, even within the same cow. Continue to clean the teat end until 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 accidentally 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.

matically be sent to your veterinarian. If you would like to receive a copy of the results directly or have a copy sent to another consultant or advisor, you must specify who and where additional copies should be sent.

University of Kentucky Livestock Disease Diagnostic Center Pricing	
Initial charge (accession fee)	\$10
General mastitis culture	\$6
Individual antibiotic susceptibilities	\$5
Maximum charge (for a single submission of samples)	\$35
Note: Prices updated July 2009.	

This fee schedule is subject to modification. Additional fees are charged when testing for mycoplasma. Your veterinarian may charge an additional fee for handling and processing the samples. The current fee schedule may be found at <http://www.lddc.uky.edu>.

Shipping Address

University of Kentucky
Livestock Disease Diagnostic Center
1490 Bull Lea Road
Lexington, KY 40511

References

- National Mastitis Council (1990). Procedures for collecting milk samples. <http://www.nmconline.org/sampling.htm>.
- Ruegg, P.L. 2005. Taking sterile milk samples. http://www.uwex.edu/milkquality/PDF/Vol_3_pdf/Pg_3_57-58_sterile_milk_samples.pdf.

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