Mastitis, or inflammation of the mammary gland, is a production-limiting infection that remains one of the most common and costly diseases in dairy herds. More than 200 different pathogens have been found to cause mastitis in dairy cattle (Blowey and Edmondson 2010). Due to the high treatment costs associated with mastitis, farmers may find value in identifying these mastitis-causing pathogens and then determining the appropriate management decision such as treatment, culling, segregation, and prevention. Milk culturing can be an effective tool for isolating and identifying organisms as well as for providing a snapshot of a herd’s mastitis pathogen status.

Because the environment of the dairy cow will be contaminated with a variety of different pathogens, it is important to follow aseptic technique when sampling milk to ensure the accuracy of milk culture results. Contaminated samples will only lead to misdiagnosis, confusion, and frustration. Storage and handling of the samples is just as important because improper cooling can allow contaminating organisms to overgrow the mastitis-causing pathogen, leading to inaccurate results. Furthermore, aseptic technique should be followed during intramammary infusion or treatment of mastitis to prevent the introduction of other mastitis-causing pathogens into the mammary gland. Some mastitis-causing pathogens, such as yeast, mold, Prototheca spp., and Bacillus spp., are known to infect the mammary gland through dirty infusions (Hogan et al. 1999). This publication provides procedures for both aseptic milk sampling and aseptic teat infusions.

### Aseptic Sampling Technique

The following procedure was adapted from the Laboratory Handbook on Bovine Mastitis (Hogan et al. 1999).

1. Record cow ID and quarter (RF, RR, LF, LR) onto each milk sample.
2. Wearing clean gloves, use a clean towel to remove gross filth off of teats.
3. Strip a few streams of milk from each quarter and record if there are clinical signs of mastitis. Clinical signs of mastitis can include but are not limited to a watery appearance, discoloration of the milk, flakes, or clots.
4. Dip all four teats with a germicidal teat dip. Allow teat dip to stay on teats for at least 30 seconds.
5. Dry teats thoroughly with a clean towel.
6. Using cotton balls soaked in 70 percent alcohol, squeeze out excess alcohol and vigorously scrub teat ends with cotton balls. Scrub teats until cotton balls remain clean. Each cotton ball should be used on only one teat.
7. Do not touch the teat end with your fingers, and do not allow the teat end to come into contact with the cow’s legs, another teat, or tail.
8. Remove the cap from the sample tube, but do not set it down or touch the inside. Collect 3-5 mL of milk from each quarter, and recap the tube. Do not touch the tube lip with your fingers or with the cow’s teat end.
9. Immediately place tubes on ice and transport them to the mastitis culture lab (2710 Litton-Reaves Hall, Virginia Tech) within six hours. If samples cannot be transported quickly, milk samples should be frozen and remain frozen until arrival at the laboratory.

Depending on milk culture results and subsequent management decisions, treatment with an intramammary infusion might be desired. Listed below are steps to follow for aseptic teat infusions applicable for both dry cow therapy as well as intramammary mastitis treatments. Without proper sanitation, organisms present on the teat end could be forced into the mammary gland, resulting in an additional infection.

Aseptic Teat Infusion Technique
The following procedure was adapted from the National Mastitis Council’s (2006) Dry Cow Therapy fact sheet.

1. Following milking and while wearing clean gloves, clean and dry teats.
2. Dip all four teats with a germicidal teat dip. Allow teat dip to stay on teats for at least 30 seconds.
3. Dry teats thoroughly with a clean towel.
4. Using cotton balls soaked in 70 percent alcohol or alcohol wipes, vigorously scrub teat ends. Scrub teats until cotton balls or wipes remain clean. A separate cotton ball or alcohol wipe should be used for each teat.
5. Do not touch the teat end with fingers, and do not allow the teat ends to come into contact with the cow’s legs, another teat, or tail.
6. Prepare teats from the far side first and then the near side. (For example, in a parallel parlor, prepare front teats first and then rear teats.)
7. Treat quarters with an intramammary infusion in reverse order: near teats first, far teats last. Insert only the tip of the cannula into the teat end and infuse all of the contents. Do not allow the cannula to touch anything prior to infusion.
8. Dip the teats with an effective germicidal post-dip.
9. Identify and record treated cows and prevent antibiotic milk from entering the bulk tank. Follow proper treatment protocols listed on the product label or through veterinarian instructions, and determine the appropriate withdrawal period to prevent antibiotic residues from entering the bulk tank.

Quick Notes
- Milk culturing is a valuable tool in identifying mastitis-causing pathogens on a dairy farm.
- Informed management decisions on treatment, prevention, segregation, or culling can be made more effectively when using milk culture results.
- Aseptic technique is critical for preventing contamination of milk samples and ensuring accurate milk culture results.
- Proper storage and handling of milk samples is important to prevent overgrowth of contaminating organisms in a milk sample.
- Aseptic teat infusions are critical for preventing the introduction of other mastitis-causing pathogens from teat ends into the mammary gland.

References
