

MASTITIS: MONITORING



BASICS

OVERVIEW

- Milk/bulk tank samples assess udder/herd mammary health at a specific point in time.
- Studies have shown >85% correlation between the number of infectious organisms in bulk tank cultures and the number of cows infected in the herd.
- Contagious mastitis pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus* in bulk tank milk samples generally reflect infected cows if the milk sample has been collected and handled properly.
- Milk culturing allows a determination of the degree of environmental pathogen exposure.
- Environmental microorganisms such as *Streptococcus* spp., coliforms, and *Staphylococcus* spp. isolated in milk cultures (especially bulk tank samples) generally originate from the skin of cows' teats. These sample results are not a direct measure of infection but reflect the potential for cow infection. With elevated environmental organism numbers on the teat skin, there is an increased incidence of mastitic infection.
- Environmental *Staphylococcus* (coagulase-negative) in milk cultures correlate with the number of organisms on the teat/mammary skin. These culture results potentially reflect the efficacy and coverage of teat dip. Improper or absent teat dipping procedure leads to higher numbers of environmental *Staphylococcus* in individual and bulk tank cultures.
- When laboratory culture results do not agree with physical observations (e.g., DHIA records, herd history, herd somatic cell count patterns), a reassessment needs to be made of the laboratory samples and collection techniques.
- Inaccurate laboratory results may be due to sample thawing or handling procedures, improper sampling/collection techniques, or environmental organism overgrowth.
- With the exception of some mycoplasmal intramammary infections (IMI) that may originate in other body sites and spread systemically, mastitis pathogens gain entrance into the udder through the teat canal.

SYSTEM AFFECTED

Mammary

GEOGRAPHIC DISTRIBUTION

Worldwide, depending on management and environmental conditions

SIGNALMENT

Species

Lactating dairy cattle, sheep, and goats

Breed Predilections

N/A

Mean Age and Range

Any lactating animal is susceptible; however, mastitis incidence increases with age and number of lactations

Predominant Sex

Female

CAUSES AND RISK FACTORS: MASTITIS PATHOGENS

- Nearly 150 different microorganisms have been found to cause IMI.
- The majority of IMI are caused by staphylococci, streptococci, and Gram-negative bacilli.
- Other important mastitis pathogens that may cause IMI in individual herds are mycoplasmas, corynebacteria, *Trueperella pyogenes*, *Prototheca* spp. yeast, and *Nocardia* spp.
- A mammary quarter is infrequently infected with more than 1 organism at the same time.



DIAGNOSIS

DIAGNOSIS OF INTRAMAMMARY INFECTION

- Diagnosis involves identification of causative agent, usually by milk sample culture.
- Aseptic collection of milk samples is a key step for accurate diagnosis of IMI.
- Common sources of sample contamination include dirty teat ends and orifices, poor collection technique (i.e. milk touches sampler's hands during collection into a vial), and residual alcohol on teat ends.
- Contamination of milk samples may lead to false-positive results when culturing.

SAMPLE COLLECTION AND HANDLING

- Samples for culture can be collected before or after milking and cooled immediately.
- Samples should be kept refrigerated or frozen during transport. Use proper packaging.
- Deliver samples to the laboratory following collection or ship overnight.
- Milk samples should be cultured as soon as possible after collection.
- Most mastitis pathogens survive refrigeration at 4-8°C for several days or freezing for several weeks.
- Freezing samples for more than 2 weeks may reduce the sensitivity for isolation of some mastitis pathogens if their original concentration in the milk sample was too low.

LABORATORY PROCEDURES

- Blood esculin agar is a general growth medium for isolation of mastitis pathogens.
- Selective media for streptococci (Edwards or TKT agars), staphylococci (Vogel-Johnson or Mannitol Salt agars), and Gram-negative bacilli (MacConkey agar) may be inoculated to enhance the recovery of those organisms.
- Bi-plates (blood esculin and MacConkey agars) and tri-plates (blood agar, TKT, and MacConkey agars) can be used to identify specific pathogen groups.

- Usually, a 0.01 mL aliquot of the milk sample is streaked in a fashion that allows the development of well-isolated microbial colonies.
- Plates are incubated aerobically at 37°C and visually examined after 24 and 48 hours of incubation for the presence of bacterial colonies.
- Culturing for the presence of *Mycoplasma* requires a special medium and incubation of plates at 37°C for 7 to 10 days with 90% humidity and 10% CO₂.
- Low number of organisms in a milk sample, inadequate handling, or improper culture procedures in the laboratory may lead to false-negative results.
- Organisms may be presumptively grouped based upon colonial morphology and hemolytic patterns of colonies on blood agar.
- Presumptive grouping should be confirmed by Gram staining or potassium hydroxide (KOH) testing before proceeding with further identification tests.
- Identification of pathogens is performed to the extent that is useful in identifying possible sources of infection and establishing effective mastitis control management.

CULTURE OF MILK SAMPLES

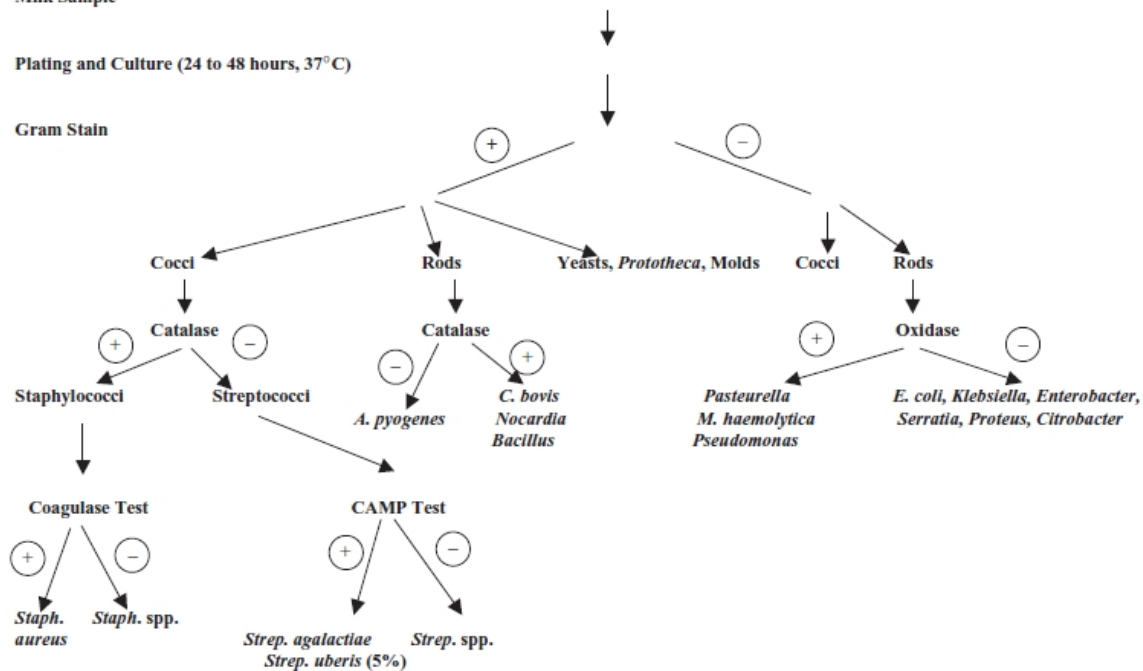
- Culturing bulk tank milk is a good screening procedure for herd monitoring of *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Mycoplasma* spp. and for determining the possible source or sources of elevated bacteria counts in a herd.
- Bulk tank culture results should not be used as a stand-alone diagnostic procedure.
- Culturing string samples provides information on individual groups of cows and is very helpful in tracing specific herd mastitis problems and evaluating cleanliness of cow's udders prior to milking.
- Culturing cow composite milk samples is suitable for identifying pathogens in recently calved heifers and cows; and cows with persistent high SCC, as well as for whole herd mastitis screening.
- Culturing of individual quarter samples is appropriate for cows with clinical mastitis or in the case of mastitic cows that do not respond to treatment.
- Chronically infected cows are usually intermittent shedders of mastitis pathogens during lactation and false negative cultures may occur.
- About 30% of milk samples from clinical cases of mastitis or from quarters with high somatic cell counts yield no mastitis pathogens.
- Diagnosis of udder infection based on multiple samples (3 to 7) is more reliable than diagnosis based on a single sample.
- If samples from a herd are consistently negative when cultured by routine methods, the presence of *Mycoplasma* spp. should be suspected.
- The growth on a medium of 3 or more dissimilar colony types when culturing quarter milk samples is an indication of contamination or poor sample collection technique.
- Uncontaminated milk samples from healthy glands contain very few or no organisms

General Microbiology Procedures for Diagnosis of Intramammary Infections

Milk Sample

Plating and Culture (24 to 48 hours, 37°C)

Gram Stain



MILK SAMPLE COLLECTION

Materials

- Sterile plastic tubes for milk collection (15 mL) with tight-fitting caps
- Tube racks
- Coolers
- Ice/cold packs
- Water-resistant permanent markers
- Cotton balls soaked in 70% ethanol or isopropanol (alcohol) or alcohol wipes
- Hand gloves

Procedure

1. All animals must be clearly identified by unique ear tag numbers.
2. Milking string should be sampled first; hospital string should be sampled last.
3. Greatest recovery of mastitic agents occurs when foremilk is cultured; therefore, collect milk samples prior to cow milking.
4. Label tube(s) with cow's ear tag number using permanent marker.
5. Ideally, cattle should be washed and relatively dry before sampling. Water "runoff" from wet cows can contaminate milk samples with environmental bacteria. Dry udder with individual milking towels if cows are wet.
6. Starting with teats on far side of gland, swab teat ends with individual cotton balls soaked in 70% alcohol or alcohol wipes to clean and decrease environmental contamination.
7. Open sterile milk tube. To prevent contamination with environmental bacteria, avoid touching tube cap or lips of tubes to cow or environment. Attempt to hold open tube near a horizontal position (without spilling milk sample) and hold cap pointing downward to

prevent environmental contamination. If contamination is suspected, discard tube and sample again using new, clean, sterile tube.

8. Starting with nearest teats, strip milk from teats twice to flush away contaminating environmental bacteria, and then fill tube approximately three-fourths full with milk and recap. Although small volumes of milk (1 to 2 mL) are required for culture, CMT tests (California Mastitis Test) require several mL of milk.
9. Composite samples from healthy cows: milk from all 4 quarters may be collected in 1 tube.
10. Hospital string/mastitis suspects: collect milk from individual quarters into individual tubes; label accordingly.
11. Sample collectors should wear disposable gloves for each cow. Ideally, hands should be washed or treated with antimicrobial substance and dried between cows to prevent spread of mastitis agents.
12. Place samples in racks labeled with milking string number. Place samples in cooler with sufficient ice/cold packs to keep samples cold until refrigerated/reaching laboratory. If samples are left at room temperature for extended periods, overgrowth with contaminating environmental bacteria may occur.
13. Bulk tank samples should also be collected in labeled tubes and sent to laboratory.
14. Samples should be refrigerated and delivered as soon as possible to laboratory.
15. Samples may be frozen. Somatic cell counts cannot be determined for frozen milk samples, but CMTs can be run on thawed milk. Repeated freeze-thaw cycles will reduce recovery of mastitis organisms and should be avoided.
16. Biohazard waste (paper towels, cotton balls, alcohol wipes, used contaminated tubes, gloves) should be disposed of appropriately (ideally incinerated). Contaminated articles could act as fomites and transmit mastitis agents to uninfected cows. Contaminated fomites could aid transmission of zoonotic agents to humans (i.e., *Prototheca* spp.).

THE USE OF BULK TANK CULTURES IN HERD MONITORING

Sample Collection and Handling

- Multiple samples collected over several days produce more consistent results.
- Multiple daily milk sampling is necessary to overcome the variability in microbial shedding that occurs with some organisms.
- Samples need to be taken from the top of the tank because bacterial growth tends to occur around the outlet valve. Should a sample be removed from the bottom of the tank, a large quantity of milk should be released through the opening before the bulk tank sample is taken.
- Milk samples should be frozen immediately and kept frozen until they are cultured. Samples that have not been frozen, or those which have thawed before use, are of no diagnostic value.
- Ship samples to the diagnostic laboratory in a foam lined/insulated container with sufficient ice/cold packs to allow the milk samples to arrive partially frozen.

Procedures for Collecting Bulk Tank Milk Samples

1. Collect samples 4 to 5 days in a row. The use of a multiple day sampling procedure is more accurate than single-day sampling.
2. Agitate/stir the tank well before sampling milk.
3. Using a sterile dipper, syringe, or test tube, remove the milk sample from the top of the bulk tank. Fill the collection tube one-half to three-quarters full. This will allow for expansion of the milk during the freezing process.
4. Freeze samples immediately. Delaying the sample freeze time will allow bacteria to grow, potentially giving false microbial growth results.

PREVENTION/AVOIDANCE

- Use proper milking technique (disposable gloves, examine udder and foremilk for signs of mastitis, apply pre-milking dipping solution, ensure udder is clean and dry, use individual towels to dry teats)
- Ensure milking equipment is functioning properly
- Apply post-milking teat disinfection
- Use dry cow antibiotic therapy
- Identify clinical mastitis cases promptly and implement therapy
- Cull chronically infected animals



MISCELLANEOUS

ZOONOTIC POTENTIAL

Contaminated fomites could aid transmission of zoonotic agents to humans (i.e., *Prototheca* spp.).

BIOSECURITY

Animals entering the herd should be examined and herd of origin history should be discussed to limit the possibility of introducing contagious mastitis pathogens into the herd. Upon calving, these animals should have a composite milk sample(s) taken for culture.

SEE ALSO

- Mastitis: Bulk Tank Monitoring and Somatic Cell Counts (www.fiveminutevet.com/ruminant)
- Mastitis: Milking Procedures and Teat Disinfection (www.fiveminutevet.com/ruminant)

ABBREVIATIONS

- CMT = California Mastitis Test
- DHIA = Dairy Herd Improvement Association
- IMI = intramammary infections
- SCC = somatic cell count

Suggested Reading

- Britten, A.M. 2012. The role of diagnostic microbiology in mastitis control programs. *Vet Clin North Am*; 28(2):187-202.
- Hogan, J.S., González, R.N., Harmon, R.J., Nickerson, S.C., Oliver, S.P., Pankey, J.W., Smith, K.L. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Inc., Madison, WI.
- Morin, D.E., Petersen, G.C., Whitmore, H.L., Hungerford, L.L., Hinton, R.A. 1993. Economic analysis of a mastitis monitoring and control program in four dairy herds. *J Am Vet Med Assoc*. Feb 15; 202(4):540-8.
- Sears, P.M., González, R.N., Wilson, D.J., Han, H.R. 1993. Procedures for mastitis diagnosis and control. *Vet Clin North Am*; 9:445-468.

- Seegers, H., Fourichon, C., Beaudeau, F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet Res.* Sep-Oct; 34(5):475–91.
- Vangroenweghe, F., Dosogne, H., Mehrzad, J., Burvenich, C. 2001. Effect of milk sampling techniques on milk composition, bacterial contamination, viability and functions of resident cells in milk. *Vet Res.* Nov-Dec; 32(6):565–79.
- Wilson, D.J., Das, H.H., González, R.N., Sears, P.M. 1997. Association between management practices, dairy herd characteristics, and somatic cell count of bulk tank milk. *J Am Vet Med Assoc.* May 15; 210(10):1499–502.

Author Maria Prado

Consulting Editor Kaitlyn A. Lutz

Acknowledgment The author and Book Editors acknowledge the prior contribution of Ruben González and Karen Carberry-Goh.