

Tennessee Quality Milk Initiative

W181

Mastitis Culturing Programs

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Implementing a culturing program to identify mastitis pathogens is the second step to troubleshooting mastitis. Many different culturing programs have been implemented, with varying levels of success. It is impossible to prescribe one program that will work on every farm. The best program for your farm should be developed with your udder health goals and objectives in mind. This publication will outline different components of culturing programs and discuss advantages and disadvantages of each.

Bulk Tank Milk Culturing (BTMC)

Culturing bulk tank milk should play a surveillance role in every dairy's overall culture program. BTMC can supply two important types of information: I) the presence or absence of a bacterial group and/or 2) the identification of predominant bacterial groups. However, mastitis detection through BTMC has limitations that you must thoroughly understand before adopting this practice.

BTMC should be performed on a *regular and frequent basis*. The more often bulk tank milk is analyzed, the more useful the results. Samples taken over consecutive days are best. It would be too costly to sample each tank. However, routine BTMC could be easily incorporated into a weekly, bi-weekly or monthly herd-health check. Extreme caution should be used when interpreting results from a single sample, as cows may shed bacteria intermittently or at levels too low to be detected by conventional lab methods. Interpreting results from a single sample may lead you down the wrong path.

Interpretation of BTMC samples can be difficult because I) relative numbers of pathogens are poorly related to infection prevalence and 2) sources of microorganisms (other than contagious pathogens) are not indicated. BTMC has very limited value for determining presence of environmental mastitis, because environmental-type bacteria could have originated from cows with environmental mastitis or be due to contamination of the bulk tank.

BTMC is an instrumental surveillance tool for identifying the presence of contagious-type pathogens. If Strep. ag., Staph. aureus, Corynebacterium bovis or Mycoplasma spp. is present in one BTMC sample, it is clear evidence that at least one cow in the herd is infected with contagious mastitis. However, if these pathogens are not present in a single sample, it is not a guarantee that all of your cows are free of these pathogens. Strep. ag is shed in very high numbers by infected cows. In fact, cows shedding high numbers of Strep. ag can actually influence Standard Plate Counts (SPC). Staph. aureus, on the other hand, is shed in very low numbers and may not be detected in a single sample. To determine if there are contagious pathogens in your herd, sampling your bulk tank on a regular basis is a must. Keep in mind that you may have to specifically request Mycoplasma spp. testing because it is not plated in the lab in the same manner as other cultures.

Culturing Clinical Cases

All dairy farms should sample cows with clinical mastitis before administering antibiotic treatment. Although results from clinical cases will not likely be available for determining treatment for that individual case, samples gathered over time will help you gain a better understanding of your farm's pathogen profile over the course of seasons, the incidence of pathogen-specific infections, and deficiencies in your herd preventative program. For example: if 10 of the last 12 clinical cases were the result of a coliform infection, it is likely that the next clinical case with similar characteristics is also a coliform infection. With that knowledge, informed decisions for treatment can be made.

The greatest disadvantage of using this approach alone is that subclinically infected cows may never be cultured or treated appropriately. Typically, most clinical cases of mastitis are due to environmental pathogens. Therefore, they are more likely to be identified using this method. Cows with contagious mastitis may not manifest clinical signs and are less likely to be identified through this sampling program.

A typical recommendation for culturing all clinical cases:

- 1. Sample the infected quarter before antibiotic treatment.
- 2. Freeze all samples.
- 3. Send a monthly batch of samples to the laboratory for analysis.

Periodic Culturing of All Lactating Cows

Collecting composite milk samples (i.e., from all quarters) from every lactating cow on the same day will allow you to estimate the prevalence of major pathogens in your herd and may identify individual cows infected with contagious pathogens. However, because of herd variation over time, extreme caution should be taken when interpreting results of a single whole-herd culture. For this type of approach to be effective in determining the prevalence of and/or verifying the spread of contagious pathogens, it must be performed routinely. Repeated culturing of chronically infected cows is not warranted, but all other cows need to be re-sampled at least quarterly. If considering this type of approach for your culturing program, schedule your sampling with the laboratory in advance to ensure samples can be analyzed promptly.

Whole-herd sampling has several disadvantages. Composite samples can identify subclinically infected cows but cannot identify which quarter(s) are infected. Additional tests, such as the California Mastitis Test (CMT), individual quarter somatic cell count (SCC) or quarter culturing will need to be performed. Additionally, research to determine how frequently whole-herd sampling should be performed is lacking. One Ontario study suggests that even a four-month interval between herd cultures may not be frequent enough for early detection of cow-to-cow spread of *Staph. aureus* (Kelton & Godkin, 2000). Finally, the greatest disadvantage of this approach is the cost of culturing an entire herd several times throughout the year. Whole-herd culturing would only be economically feasible for smaller herds.

Strategic Culturing of Late Lactation Cows and Early Lactation Cows

Collecting composite samples from cows at these critical stages in lactation can reveal if your dry cow therapy is effective and/or if new infections are occurring during the dry period due to dry and transition cow environments. For example, if Cow #851 has a negative culture at dry-off, but tests positive for an environmental *Strep*. infection shortly after calving, she likely contracted that infection in the dry/transition cow housing or the calving area. As another example, if Cow #1272 tests positive for *Strep*. ag. at dry-off, but tests negative for pathogens shortly after calving, the dry cow therapy was successful.

At this time, it is not clear how soon after calving samples should be taken to best reflect the true infection status of the fresh cow. It is known that SCC is highly variable for up to one week after calving, but the sensitivity and specificity of bacteriological culture of colostrum and/or early lactation milk have not been studied. Our best recommendation at this time is to sample after colostrum has cleared. For some cows, this may occur within two days. For other cows, it may take longer. The disadvantage of waiting even a few days after calving is that the source of new infections could be due to dry cow environment, calving area environment or early lactation cow environment. You must investigate each area thoroughly to determine the most likely source of new infections.

There are additional downsides to this approach that you should consider. The relatively low sensitivity of a single milk culture for detecting *Staph. aureus* is a concern, and serial sampling over several days should be considered. Additionally, the costs of sampling each cow a minimum of two times each lactation may be staggering for larger herds.

Strategic Culturing of Herd Additions

Most biosecurity programs for dairy herds recommend that purchased lactating cows be cultured and segregated from the rest of the herd for some time after arrival. This practice is an important component of a mastitis control program, but it is not often followed. If you purchase springing heifers, dry cows or lactating cows and do not culture these animals, you are putting your entire herd at a very high risk of contracting contagious mastitis. Many times, herd SCC history is available before purchase. Unfortunately, purchasing cows from herds with a "low SCC" may give you a false sense of security. Herds that have a *Staph. aureus* problem may have an overall low SCC.

If you purchase animals, consider adopting some of the following recommendations when possible:

- 1. If purchasing springing heifers or dry cows, insist that the bulk tank of the selling herd be cultured for contagious pathogens multiple times before the sale date. You might have to specifically request a *Mycoplasma spp.* test.
- 2. When purchasing lactating cows, request that individual cows be cultured multiple times for contagious pathogens (including *Mycoplasma spp.*) prior to sale date.
- 3. If culturing prior to purchase is not possible, gather as much information on the selling herd or animal as possible (herd SCC, individual cow SCC from previous or current lactation, clinical mastitis records, etc.). In addition, culture individual cows over consecutive days and segregate these animals until results are available.

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All herds that purchase animals should adopt these practices. Failure to do so could lead to the introduction of very costly diseases into your herd.

Strategic Culturing of High SCC Cows

If individual cow SCC data are available through DHIA services or by other means, sampling cows with an elevated SCC may be a useful tool for your herd. However, if relying on *monthly* SCC data, it is important to remember there is a lag time between the time of infection and the increase in a composite SCC. In other words, a cow could have been infected yesterday, but SCC may not be elevated today. It will likely be another month before the next SCC data is available, and it may or may not be elevated at that time.

There are two primary situations in which culturing high SCC cows should be considered: I) to identify new contagious infections and 2) in response to an immediate need to lower infection rate. In the first situation, contagious mastitis is typically a subclinical disease. It will eventually raise a cow's SCC, but it may not result in clinical mastitis. Careful monitoring of individual cow SCC during lactation and culturing when SCC is elevated may provide an opportunity to identify newly infected cows. The second situation is when herds are at risk or have exceeded the regulatory SCC limit, and immediate action is needed. Culturing cows with a high SCC will provide valuable information that can be used to determine treatment regimens for problem cows. In some cases, the high herd SCC may only be due to a few individual cows. In other cases, there may be a significant number of cows with high SCCs contributing to the bulk tank. Pulling a large number of cows out of production at the same time for treatment may seem like an extreme approach. However, the consequences of not taking immediate action could lead to large penalties or losing your market due to exceeding the regulatory limit. This approach may also be warranted if your milk purchaser has adopted a more stringent SCC limit (lower than the regulatory limit) and/or a SCC premium program is available.

Collecting, Storing and Shipping Culture Samples

The most critical aspect of any culturing program is the collection, storage and shipping of samples. If strict protocols are not followed, results obtained may cause confusion and lead you down the wrong path, or the results may simply be worthless. A very frustrating result of culturing is a laboratory report of "Contaminated Sample." The frustration is two-fold. First, the results are of no use. Secondly, you have paid money for useless results. The old saying of "garbage in equals garbage out" should be the motto of every farm's culturing program, and great care should be taken with each sample to ensure meaningful results. The procedures outlined below for collecting, storing and shipping bulk tank milk samples and individual cow samples have been adopted by the Tennessee Quality Milk Laboratory.

Collecting a Bulk Tank Milk Sample

- 1. Agitate bulk tank milk. Turn on the agitator and run for at least 10 minutes. Proper agitation is necessary to ensure all of the milk in the tank is represented in the sample.
- 2. Check bulk tank milk temperature. While agitating, check the temperature of the milk in the tank. It should be 40 degrees F or lower.

Collecting a Bulk Tank Milk Sample - Continued

- 3. Label sample tubes. Record the date, time, milk temperature and farm name on two tubes. Make sure there is NO preservative in the tubes.
- 4. Take sample from top of bulk tank. Samples obtained from the outlet at the bottom of the tank give inaccurate results.
 - a) After agitation, open the lid of the bulk tank.
 - b) Use a sterile dipper.
 - c) Rinse the dipper twice in the milk
 - Fill the dipper a third time and remove it from the tank
 - Fill sample tubes at least 3/4 full.
 - Do not fill tubes over the open tank
 - Do not touch the inside of the cap, the tube opening or the inner surface of the tube.
 - d) Collect two tubes of milk to ensure there is enough milk for all of the tests.
 - e) Discard any unused milk.
 - f) Close the bulk tank lid.
- Refrigerate bulk tank milk samples. Keep samples refrigerated until they are sent to the laboratory. DO NOT FREEZE SAMPLES. This will make somatic cell count analysis and Mycoplasma culture impossible.
- 6. Complete the sample submission form. Complete all of the necessary submission forms.
- 7. Submit for analysis. Samples should be sent to the lab <u>AS SOON AS POSSIBLE</u> and within 48 hours of sampling to ensure accuracy of results. For most laboratories, samples received during the first part of the week (Monday-Wednesday) will be plated shortly after arrival. If the laboratory is not open on weekends, samples received on Thursday and Friday will likely be held until the following week. The Tennessee Quality Milk Laboratory is closed on weekends.

Collecting Quarter Samples

- 1. Label sample tubes. Sample tubes should be labeled with a **waterproof** marker identifying cow and quarter **before** starting sample collection.
- 2. Prepare udders and teats. A clean, dry teat end goes a long, long way towards preventing contaminated samples!
 - a) Brush or wipe any bedding, debris or manure off the teats and base of the udder. If teats and udder are caked with wet manure, wipe as much off as possible with paper towels before washing teats with as little water as possible and dry completely.
 - b) Clean teats with a disinfectant solution. Teat disinfectants are a good choice.
 - c) Dry teats completely with a clean paper towel.
 - d) Strip and discard the first stream of milk.
 - e) Starting with the teats on the far side of the udder, scrub each teat end vigorously. Scrubbing of each teat end should continue until a new surface of the alcohol pad remains clean. Use separate alcohol pads on each teat. (A moistened, but not completely wet, cotton ball with 70 percent ethyl alcohol can be used if an alcohol pad is not available.)
 - f) If you touch a teat end, clean it again with an alcohol pad.

Collecting Quarter Samples - Continued

- 3. Collection of samples. Use strict aseptic procedures when collecting milk samples.
 - a) Remove the cap from the sample tube **without touching** the inside of the cap, the tube opening or inner surface of the tube. Do not allow the cap or tube opening to touch teat ends. Touching any of these will contaminate the sample.
 - b) Start with teats closest to you, and then sample teats on the far side of the udder.
 - c) Hold the tube at an angle and direct the milk stream into the tube. Fill the tube 1/4 to 1/3 full typically one stream of milk. There is no need to fill the tube completely.
 - d) Replace cap on tube as soon as sample is collected.
 - e) Dip teats with a post-milking teat disinfectant if the cow is not going to be milked immediately.
- 4. Keep samples cold. If collecting multiple samples, place samples in cooler (with ice or cold packs) or in refrigerator until all samples are taken.
- 5. Freeze samples. After sample collection is completed, samples should be stored in a freezer (at -20 degrees F) until sent to the laboratory. If submitting individual cow sample for Mycoplasma, DO NOT freeze the sample.
- 6. Complete the sample submission form. Complete all of the necessary submission forms.
- 7. Submit for analysis. As long as samples are kept frozen, they can be stored for up to one month. However, usefulness of results may decline as time passes. Ship samples on ice packs.

Packaging and Shipping Milk Samples

(Adapted from the Tennessee Quality Milk Laboratory Web site (www.tqml.utk.edu))

Shipping of milk samples must follow DOT and IATA regulations that govern the shipping/ transport of biological materials. Milk samples must be shipped in a "triple-package system." Failure to package and label correctly may result in samples not being delivered or returned by the carrier. The Tennessee Quality Milk Laboratory has sampling packs available that include all necessary materials to triple pack samples for shipment.

- 1. Primary Container. This is the sample container (tube, vial or whirl pack).
 - It must be leak-proof when closed completely.
 - The primary container must be placed in a secondary container.
- 2. Secondary Container. This is a sealable bag (ex: a Zip-Lock™ bag).
 - Put sample tubes in a sealable bag.
 - Pack paper towels around the sample tubes in the sealable bag. There must be enough paper towels to absorb all of the contents in the tubes if a leak occurs.
 - The secondary container must be placed in a tertiary container.
- 3. Tertiary Container. This is a Styrofoam[™] cooler.
 - Place sealable bags in cooler.
 - Place cold packs around sealable bags. Do not put cold packs inside bags containing samples.
 - Fill empty spaces in the cooler with some type of packing material (newspaper, Styrofoam™ pellets or bubble wrap) to prevent the contents from moving around.
 - Do NOT ship unprotected Styrofoam $^{\text{TM}}$ coolers, as they are easily broken or punctured. They must be placed inside a cardboard box.

Packaging and Shipping Milk Samples - Continued

- 4. Outer Container. This is a cardboard box.
 - Place the submission form in a sealable bag between the cooler and the cardboard box.
 - Label the box with:
 - a) "Exempt Animal Specimens" label.
 - b) Sender's return address.
 - c) Lab address.
- 5. Ship. Send entire package to the lab as soon as possible using UPS or FEDEX. Samples should arrive at the lab within 1-2 days of shipping. Overnight shipping will ensure that samples arrive at the correct temperature. Delaying the samples' arrival at the lab may affect the accuracy of analysis. Do not ship samples to arrive on weekends.

Interpreting Culture Results

Once samples have been shipped to the laboratory for analysis, you must endure the wait. For most samples, definitive results can be obtained within 72 hours. Some labs may offer preliminary results within as few as 24 hours after samples have been plated, followed by definitive results after 72 hours of growth. Exceptions are samples plated for *Mycoplasma spp*. Most labs use a seven-day standard for *Mycoplasma spp*. testing. However, in low *Mycoplasma spp*. prevalent herds, it may take as long as 14 days for definitive results because of very low numbers of organisms in the sample. For most producers, it is the frustration of the waiting time that discourages them from adopting a culturing program. Results are wanted immediately to determine the best course of treatment for a cow with clinical mastitis. It is vital to remember that a culturing program is designed to give you *knowledge* so that you can make informed decisions on *future* cases of mastitis, not the cow that currently has clinical mastitis.

Bulk Tank Milk Culture (BTMC) Reports

Results from BTMC can be very confusing. There will likely be several different organisms listed, with each detected at different levels. This may lead you to believe your cows are infected with everything. Most of the time, this is not the case. You must remember that bacteria can enter the bulk tank independent of cow infections. Milking parlors and bulk tank rooms are not sterile environments. Even herds with the most stringent milking hygiene and equipment-cleaning processes will likely have contamination in the bulk tank. The key to understanding BTMC reports is knowing which organisms are significant and at what levels.

The primary purpose of BTMC is to screen the herd for contagious pathogens. Therefore, the first question to ask is whether or not the BTMC sample is positive for *Strep. ag*, *Staph. aureus*, *Corynebacterium bovis* or *Mycoplasma spp.* If the report indicates the presence of any contagious pathogens, even at very low levels, it is a clear indicator of infected quarters within the herd. Unfortunately, the level detected cannot predict the number of quarters infected. Sampling of individual cows would need to be performed. If a single report does not reveal the presence of contagious pathogens, it is not a guarantee that the herd is free of these pathogens. It is vital that BTMC is performed routinely, especially for herds that purchase animals.

Bulk Tank Milk Culture (BTMC) Reports - Continued

In addition to contagious pathogens, your report will likely list an array of environmental pathogens isolated from the sample. Unfortunately, there is no true method available to distinguish between environmental pathogens originating from infected cows and those due to bulk tank contamination. Low levels of individual environmental pathogens could originate from either. Moderate levels could be from one or both sources. The real advantage of testing for environmental pathogens is that very high levels are a strong indicator of poor milking hygiene or deficiencies in equipment cleaning. An exception may be when a large number of cows with clinical mastitis are milked into the bulk tank. Unfortunately, the number of colony forming units (cfu) that constitutes very high levels is different for each pathogen and not easily remembered. Ranges for different bacteria are depicted in Table I. If BTMC reports indicate high or very high levels of environmental pathogens, investigate milking time hygiene and equipment cleaning.

Table 1. Interpreting Environmental Pathogen Levels in BTMC Reports.

Туре of Bacteria	Low Levels	Moderate Levels	High Levels	Very High Levels	
Coliforms	<100	100-400	400-700	>700	Total
E. coli	<10	10-100	100-400	>400	Coliforms
Staphylococcus sp.	<300	300-500	500-750	>750	
Enterococcus sp.	<100	100-700	700-1200	>1200	Environmental
Streptococcus sp.	<700	700-1200	1200-2000	>2000	Streptococcus

Source: AgSource Cooperative Services, Food & Environmental Laboratories

Table 2. Bacterial Species Isolated from BTMC

Type of Bacteria	Species (Species indicated in BOLD associated with subclinical & clinical mastitis)			
Coagulase-negative Staphylococci	S. caprae, S. chromogenes, S. cohnii, S. epidermidis, S. hominis, S. hyicus, S. intermedius, S. lentus, S. simulans, S. warneri, S. xylosus			
Streptococci & Strep-like organisms	S. mutans, S. salivarius, S. alactolyticus, S. bovis, S. equinus, S. uberis, E. faecalis, E. faecium, E. saachronlyticus, E. avium, E. durans, E. hivae, E. malodoratus, S. agalactiae, S. dysgalactiae, S. equi, S. equisimilis, S. zooepidemicus, S. downei, S. acidominimus, S. thermophilus			
Coliforms	Escherichia coli, Citrobacter fruendii, Klebsiella spp., Enterbactor spp.			
Gram-negative non-coliforms	Acinetobacter, Hafnia, Moraxella, Pseudomonas, Serratia			

Source: B. Jayarao, Extension Veterinarian, PSU

Individual Cow Culture Reports

When culturing a single milk sample, there are four possible results:

- 1. Correct Result. Bacteria isolated in the sample are truly the cause of infection.
- 2. False-Positive. A pathogen is isolated, but the quarter is not truly infected. Such results occur due to contamination at some point during sample collection and/or processing. If a single milk culture sample is the only criterion for determining infection status, a false-positive will be interpreted as an infected quarter. Using individual cow SCC coupled with strict aseptic sampling techniques will reduce over-interpretation of false-positives.
- 3. False-Negative. No growth occurs, but the quarter is truly infected. Approximately 30 percent of culture samples will result in no growth. There are several reasons why this occurs.
 - A) A certain number of organisms in the sample are necessary for detection. The number of bacteria shed by animals can vary greatly, even during clinical stages of disease. When the sample was taken, the number of bacteria being shed may have been too low for detection. Additionally, the dilution factor of composite samples versus individual quarter samples can contribute to low levels of organisms.
 - B) Organisms may no longer be present due to phagocytosis of somatic cells. Clinical signs may be due to damage caused by the pathogen.
 - C) Antibiotics previously used may have reduced organisms to undetectable levels. On occasion, visible signs of infection disappear after treatment but reappear a short time later. This often entices producers to take a sample. As a general rule, it is best to wait two weeks after antibiotic treatment before sampling. The antibiotic may have worked, and visible signs are due to endotoxins or other damage caused by the pathogen. Alternatively, the antibiotic may suppress, but not kill, pathogens to a level too low for detection.
 - D) Storing samples may reduce numbers of viable organisms below detectable levels. Freezing individual cow samples and submitting weekly, bi-weekly or monthly batches is a common recommendation. This is for ease of submission and reduction in shipping costs, but it comes at a price. Most bacteria are not affected by freezing for several weeks. However, freezing samples may reduce the likelihood of isolating some bacteria, such as *Mycoplasma spp.*, *Nocardia spp.*, *E. coli* and other coliforms.
- 4. Contaminated Sample. When culturing results in the growth of three or more pathogen types, results are impossible to interpret, and the sample will be recorded as contaminated. At low levels of contamination (i.e., two pathogen types isolated), both types may be recorded. Unfortunately, it is difficult to interpret this as well. You may receive a report listing several bacteria that were isolated, but they are likely the same type of bacteria. For example, individual bacteria isolated may all be Coagulase-negative Staphylococci (CNS). The report may list all of the bacteria individually or may record them as CNS.

In addition to false-positives, false-negatives and contaminated samples, another confusing result may occur, the *odd-ball pathogen*. When culturing a large number of animals, occasionally a specific pathogen may be isolated from only one sample out of many. Serratia, Prototheca, Pseudomonas and many other environmental bacteria can cause mastitis infections. If they only appear infrequently, it is likely due to contamination of the sample. However, if the *odd-ball pathogen* appears on several occasions, take it seriously.

Antibiotic Sensitivity Testing

The purpose of requesting antibiotic sensitivity testing on individual cow culture samples is to determine the best antibiotic therapy for mastitis caused by that specific pathogen. Some antibiotics are only effective against certain types of pathogens, whereas others are effective against many types of pathogens. Even within the broad-spectrum antibiotics, some are more effective against individual pathogens than others. Having an antibiotic sensitivity test performed on a significant number of individual cow cultures is the best method for determining which antibiotics should be used for future cases of mastitis. Unfortunately, farm results do not always mimic lab results. If antibiotic sensitivity testing indicates that Antibiotic X is the best choice for treating your cows, it is not a guarantee that you will cure all similar cases of mastitis with Antibiotic X. In the lab, pathogens isolated from cultures are directly exposed to antibiotics in a controlled environment. In the udder, pathogens can evade antibiotics due to the extremely large surface area of the milk duct system. The prescribed dosage of antibiotic may simply not reach the site of infection or be present in significant quantities, in which case an increased dose or extended doses may be warranted. Caution: treating cows off-label can only be performed under the direction of your herd veterinarian. Also, changing the dosage may increase residual withdrawal time and caution should be taken before milk is placed in the bulk tank. Although antibiotic sensitivity testing has its limitations, it is the best technology available at this time for determining the most effective antibiotic therapy.

Conclusions

Adoption of a culturing program is a vital step in troubleshooting mastitis. By knowing which pathogens are causing mastitis, you can target management decisions for greatest impact. Without that knowledge, successful treatment is largely due to luck, rather than science. The best culturing program for your farm should be tailored from the components discussed in this publication with your udder health goals and objectives in mind. Experienced professionals and herd veterinarians can help you develop a culturing program and interpret results.

References

Kelton, D.F., and M.A. Godkin. 2000. Mastitis Culture Programs for Dairy Herds. *Proceedings of the National Mastitis Council Annual Meeting*. p. 54-62.