National Mastitis Council

Using Bulk Tank Milk Cultures in a Dairy Practice

Introduction
The continued popularity of culturing bulk tank milk (BTM) to monitor milk quality suggests that this procedure is beneficial to dairy producers and practitioners. However, few laboratory procedures for testing milk quality are more abused and misused than BTM bacteriological culturing. Guidelines for testing procedures have not been adequately evaluated under controlled scientific conditions. Interpreting results requires that practitioners fully understand the limitations of the culturing method.

Despite the limitations cited above, many practitioners have successfully incorporated BTM culturing into herd monitoring procedures. Those who have successfully adapted a BTM culturing program into practice understand these limitations and do not go beyond the intended scope of use as a monitoring tool.

Sampling Interval
Probably the most common question regarding BTM is, "How often should we sample?". The answer is, "As often as possible!" The more often BTM is sampled, the more useful the information will be to the practitioner. Ideally each tank of milk should be sampled, but such a sampling schedule is impractical. One practice that works for many is to incorporate BTM sampling into the weekly, bi-weekly, or monthly herd health checks. Some producers collect BTM samples weekly and freeze them until the practitioner arrives for the herd health check. This integration allows the service to be offered as a total herd health program.

A common scenario is for a producer with a milk quality problem to bring in a single BTM sample for culturing. Extreme caution should be taken when interpreting results from a single BTM sample. Even if the dairy is facing down-grading, try to obtain samples from BTM over a period of time. Samples taken over consecutive days or weeks are most helpful and a clearer picture of the producer's problem will likely come into focus. Additional information including individual cow somatic cell counts, and milking systems and procedures analysis should be used when correcting deficiencies that decrease milk quality.

Methods
There is no industry standard for BTM culturing. A procedure successfully used is outlined below. This procedure or a variation of it can be adopted in most practitioner's laboratories.

1. Sample collection is critical. Agitate the milk in the bulk tank for five minutes prior to collection. Always collect the sample from the top of the bulk tank (never the outlet) using a clean sanitized dipper. Place samples on ice immediately or refrigerate them until they can be streaked for microbiological culture.

2. Samples are plated by streaking .01 ml of milk vertically the diameter of an agar plate. The milk inoculum is evenly spread over the entire surface of the plate by a back and forth motion at right angles to the central streak with the same loop used for inoculation.

3. Each sample is plated in duplicate on trypticase soy agar containing 5% bovine blood and 0.1% esculin (TBA), MacConkey agar (MAC), TKT agar, and mannitol salt agar (MSA) or Staphylococcal Medium 110 (SM).

4. Agar plates are incubated at 37 degrees C for 48 hours.

5. Counts from duplicate plates are averaged and colony forming units (CFU) per milliliter (ml) recorded as total bacterial growth (total growth on TBA); gram-negative bacteria (total growth on MAC); coliforms (lactose-positive colonies on MAC); streptococci (total growth on TKT); and staphylococci (total growth on MSA or SM).

6. Suspect *Staphylococcus aureus* colonies are confirmed by the tube coagulase test or by an anti-protein A and clumping factor latex agglutination test. Suspect *Streptococcus agalactiae* colonies are confirmed by the CAMP reaction or Lancefield group B latex agglutination reaction.
These procedures result in a semi-quantitative measure of common bacterial groups in BTM. The more diverse and selective the media used for BTM culturing, the more likely that bacterial contaminates will be correctly identified and enumerated.

**Results**

Bacterial isolates from BTM are typically a heterogeneous mixture of various taxonomic and ecological groups. Theoretically, any bacterial isolate from BTM could arise from an intramammary infection (IMI). The probability of an isolate originating from an IMI is dependent upon the bacteria. A primary function of BTM culturing is to determine if a herd is positive for the contagious pathogens *Staphylococcus aureus* and *Streptococcus agalactiae*. The presence of these pathogens in BTM almost always indicates the presence of infected quarters in the herd. Characteristics of some common BTM isolates are summarized below.

**Streptococcus agalactiae**

The only reported reservoir for *Streptococcus agalactiae* is infected udders. *Streptococcus agalactiae* presence in BTM is due exclusively to the shedding of bacteria in milk from infected quarters. *Streptococcus agalactiae* infected quarters significantly contribute to both elevated BTM standard plate counts (SPC) and somatic cell counts (SCC). Bulk tank milk samples from *Streptococcus agalactiae* infected herds frequently contain high bacterial counts (20,000 to 100,000 or greater CFU/ml) due to cows shedding this organism. An effective mastitis control program, emphasizing teat dipping and total dry cow therapy, will eradicate this microbe from a herd within 2 to 3 years, if the herd remains closed. *Streptococcus agalactiae* eradication is an attainable goal.

**Staphylococcus aureus**

Contrary to *Streptococcus agalactiae*, *Staphylococcus aureus* infected quarters shed bacteria in low numbers. *Staphylococcus aureus* seldom causes high bacterial counts in BTM. However, a strong correlation exists between number of *Staphylococcus aureus* in BTM and BTM somatic cell counts in *Staphylococcus aureus* infected herds, provided *Streptococcus agalactiae* is not present. Presence of *Staphylococcus aureus* in BTM results from the same management inadequacies as those responsible for *Streptococcus agalactiae*. Implementing and maintaining an effective mastitis control program will generally reduce the level of *Staphylococcus aureus* infected quarters to <1% within a herd. An attainable goal is < 50 CFU/ml in BTM.

**Staphylococcus species**

*Staphylococcus* spp. (staphylococcal species other than *Staphylococcus aureus*) are teat skin flora and often are the bacterial group most frequently isolated from infected glands. High *Staphylococcus* spp. counts in BTM may indicate poor udder preparation and teat sanitation. Intramammary infections with this group of organisms usually result in only a slight elevation of quarter SCC ( <300,000/ml). Isolated instances have been noted where high standard plate counts were due to growth of *Staphylococcus* spp. in improperly cleaned milking equipment. An attainable goal for *Staphylococcus* spp. counts in BTM is <1,000 CFU/ml.

**Environmental streptococci and coliforms**

Isolating large numbers of environmental streptococci and coliforms from BTM indicates poor hygiene either during equipment cleaning and sanitation, during milking, or between milkings. These two microbial groups share common sources of contamination such as bedding, soil, manure, and water. Milking wet udders, organic soil buildup in milklines, cracked gaskets and inflations, inadequately heated wash water, inadequate cooling of milk, and IMI can all contribute to high environmental streptococcal and coliform counts in BTM. Lowering BTM counts of these bacteria usually involves evaluation of environmental situations. Environmental factors contributing to BTM counts also harbor the potential for increased IMI rates. This association was evident by the positive correlations among environmental streptococcal and coliform rates of clinical mastitis, bacterial counts in bedding, and BTM counts in a study involving nine well-managed dairy herds. Realistic goals for BTM counts of environmental streptococci and coliforms are <1,000 CFU/ml and < 500 CFU/ml, respectively.

**Interpreting BTM cultures**

The first question to ask when interpreting BTM cultures is whether or not the samples are positive for *Streptococcus agalactiae* or *Staphylococcus aureus*. Isolating either of these contagious mastitis pathogens from BTM means that at least one quarter of one cow that was milked into the bulk tank had an IMI with that pathogen. However, BTM counts can not be used to predict the number of quarters infected within a herd. In addition, negative culture results do not necessarily mean that the herd is negative for IMI caused by these pathogens.
Another question that can be answered is, "What are the predominant bacterial groups in the BTM sample?". For example, if *Streptococcus agalactiae* is the predominant bacteria in BTM having high standard plate counts, the first area of improvement for BTM quality could be to reduce the number of quarters infected with *Streptococcus agalactiae*. On the other hand, if coliforms are the predominant bacterial group in BTM, another set of tactics must be employed to find the contamination source(s). In general, coliforms are present in very low numbers in BTM (<500 CFU/ml). High coliform counts may be associated with improper cleaning of the milking system, improper milking procedures, inadequate cooling of milk, and IMI. High counts from coliform IMI are infrequent but they do occur. This is also true for the environmental streptococci. Interpreting BTM culture results requires an understanding of the ecology of these bacterial groups and their sources for contaminating BTM.

**Summary**

Bulk tank milk culturing should be done only if the practitioner is aware of the many pitfalls associated with this procedure:

1. Bulk tank milk culturing is based upon limited scientific data; however, BTM culturing can supply two important types of data: a) presence or absence of a bacterial group; and b) identification of predominant bacterial groups in BTM. Beyond these data, assumptions must be made as to the relevance of information gained.

2. Bulk tank milk culturing is more useful in monitoring concurrent changes in conditions than as a tool to diagnose what conditions have previously changed. Many changes in management (good and bad) will be reflected by BTM cultures. Increasing frequency in which samples are taken will increase the probability of detecting these changes. Implementing changes in management based upon findings from BTM cultures should be based upon a thorough knowledge of the ecology of bacteria and supplemented with information from other records such as somatic cell counts, preliminary incubation counts, clinical mastitis incidence, etc.

3. **Most important -- BTM cultures are never substitutes for quarter milk samples.** BTM cultures are useless indicators of IMI prevalence in a herd. BTM cultures can be valuable supplements to quarter milk samples, but never a substitute for determining IMI incidence and prevalence based on quarter milk samples.

*Source: National Mastitis Council factsheet*