The devil is in the details: Opportunities and pitfalls of providing an in-clinic milk quality laboratory.

James W. Bennett, DVM, Northern Valley Dairy Production Medicine Center, Plainview, MN 55964, Bennettnvac@gmail.com, www.dairymed.com, (507) 534-4356
Abstract

Microbial culture of milk for diagnosis of pathogens has been a mainstay of mastitis control for many years. Traditionally services have been provided by outside laboratories or to some degree, by local veterinary clinics. In-clinic laboratories can perform basic microbiological testing of milk and other materials related to udder health. Such services can provide valuable information for mastitis control; however there are many pitfalls to consider when designing or operating a laboratory. Recently, increased focus on responsible antibiotic use by the dairy industry, dairy veterinarians, and society in general has created a greater need for microbial identification prior to antibiotic treatment. The aim of this article is to describe potential uses of an in-house laboratory while also describing some of the potential problems the veterinary practitioner may face. There are variety of techniques, beyond simple milk culture that can be performed in a clinic laboratory. The information provided may help practitioners provide valuable information and service to dairy producers who wish to improve or maintain udder health.

Keywords: Milk quality, udder health, milk culture, microbiological laboratory, mastitis

Introduction

According to Britten, there is an opportunity to “profoundly enhance the effectiveness of the mastitis control efforts of the dairy practitioners” by influencing the type and scope of laboratory support offered to dairy farms. Britten defines the scope of service as the “specific mastitis organism diagnostic capabilities for which the laboratory will provide.” The purpose of this paper is to identify and describe specific opportunities and pitfalls of an in-clinic lab, while at the
same time providing examples of what might be appropriate scope of service for a typical in-
clinic milk quality lab. Opinions expressed in this paper are based on the author’s experience in
designing and operating a clinic laboratory for over 30 years and on veterinary literature.

Northern Valley Dairy Production Medicine Center is a four-doctor, large animal practice in
southeastern Minnesota. Our dairy herds range in size from 50 to 3,000. All but a few are free
stall herds. We have operated our lab since the early 1980’s. At first, we performed individual
quarter or cow bacterial culture and antibiotic sensitivity. We added bulk tank culture soon after.
Individual culture was usually performed on clinical, non-responding mastitis cases, with the
goal being to determine how to treat the cow. Culture-based treatment was seldom used as part
of the initial treatment plan. Indeed, culture based therapy of clinical mastitis was not common
on farms across the US at that time. As a result, the number of individual samples submitted on
a regular basis was pretty small. Early in the 21st century we began to strongly encourage culture
based treatment, based on the work by others that a large percentage of cases on a typical dairy
would not benefit from antibiotic treatment. At the same time we began providing pathogen and
farm specific treatment protocols, because we believed they would be of value to the farms. We
also discontinued sensitivity testing because it was clear that it provided little benefit to the cow
or dairy producer. We also began to consider some limited treatment of subclinical cases.

Over time we added additional services, including: support for on-farm culture programs,
bedding, water, towel, calf milk, calf colostrum, and sanitation audits utilizing ATP swabs.

Today, about 70% or more of the cows found on dairies that treat clinical mastitis in our practice
live on farms where culture based treatment (CBT) of clinical grade 1 and 2 mastitis is
performed. A number of farms have been successful operating on-farm culture labs. Farms
deliver samples to our lab on a daily basis. We have many clients who provide bulk tank
samples monthly for bulk tank culture. Calf milk, calf colostrum, bedding, towel, and water cultures are requested mostly on as-needed basis. We do not provide PCR, standardized plate counts (SPC), pre-incubation (PI) or lab pasteurized counts (LPC), but any of all of these might be considered as opportunities by other veterinary clinicians.

We recognize that a couple big-picture pitfalls exist for an in house laboratory. One is that antibiotic treatment for clinical mastitis could become very uncommon or even eliminated in the future. While we believe that this course may not be wise, we understand that it could still happen. Another is that most of our clients could decide to operate in-house culture labs. Should that happen it would significantly reduce the supply of individual milk samples coming into our lab. Nevertheless, we have been at this for quite some time, and most clients still do not have on-farm labs.

Specific test opportunities for a clinic laboratory

Culture based therapy of clinical mastitis probably is the largest opportunity for a milk quality lab for most practitioners. For example, a practice that serves 20,000 cows with a 3% overall clinical case rate per month could potentially culture 600 samples per month. This could provide significant revenue to the practice. In the real world this degree of adoption is unlikely, but even if only 50% of cases were cultured there would still be an opportunity for significant revenue.

Providing a culture-based treatment service requires a mind shift to that of mostly finding cows *not to treat*, instead of finding cows to treat, and ways to treat them because the greatest economic advantage to the dairy is by not treating cows that are unlikely to benefit. In the author’s opinion an effective culture based therapy service requires development of organism-specific and farm specific treatment protocols. There are a few, but not many peer reviewed papers that discuss organism-specific protocols, and the veterinarian may be wise to use these to
develop treatment protocols. Different farms have different goals, for example one producer may wish to attempt to treat some Staph aureus infected cows, while another will immediately cull or segregate them without treatment; thus the argument for farm-specific protocols.

This paper will not describe, in detail, the rationale or mechanics of CBT. However various studies have shown that 50% or less of clinical mastitis cases benefit from treatment on a typical dairy.\(^8,17\) Indeed, Ruegg\(^17\) has reviewed the literature and provides the following summary, “…the overall proportion of (clinical, grade 1 & 2) cases that can be expected to benefit from ….treatment ranges from 20% to 33%”. This is the primary rationale for culture based therapy, though others, such as increased treatment effectiveness, and identification of cows carrying contagious pathogens exist as well. It is also our opinion that the veterinarian that thoroughly understands culture methods provides needed support to farmers with on-farm labs, because he or she can answer questions or provide a clinic lab as back up support for the farm lab. Indeed, lack of support is one of the primary reasons farmers discontinue on-farm culture.\(^19\)

Culture based therapy is not without pitfalls. First, the laboratory needs to provide rapid turn-around. Ideally this means before the time of day treatments are administered on the farm for the very next day. Results need not to be final by the next day, but providing rapid, preliminary results allows producers to implement treatments in a timely manner, and may decrease the resistance producers sometimes offer to delaying treatment while waiting for results. It should be noted that delay of treatment does not typically adversely affect results, however.\(^13,14\) Second, a culture based lab should, ideally operate on weekends. This standard may be difficult to accomplish in some practices. Third, veterinary advocates of CBT are advised to consider the real, on-farm effects of implementing CBT. For example: Who takes the milk sample? Where does it go next? What happens to the cow with clinical mastitis while the farm waits for results?
Where does the milk go before treatment? These and many other on-farm factors may likely determine whether a farm adopts or continues CBT. Any attempt to understand these before advocating CBT to a particular client might encourage adoption. In spite of these potential pitfalls, our practice has found that CBT injects science and rationality into mastitis treatment decisions.

Screening cows for contagious pathogens is another opportunity for an in-house lab. In our local world, isolation of Strep ag is rare. Staph aureus is still found on farms, but much less commonly and at much lower rates than in the past. This pattern is typical of dairy herds in the United States. Mycoplasma may be a more common isolate than in the past in our area and in other northern states, while Prototheca is becoming much more common in our herds. Indeed, Prototheca is the most commonly identified “contagious” pathogen in our laboratory today.

Note: our experience indicates that Prototheca should indeed be considered a contagious pathogen, even though there is little, if any, published research arguing this to be the case. Since most dairy veterinarians understand the importance of identifying cows with contagious pathogens we will not describe the rationale any further here. There are a few points to consider for veterinarians who might be thinking about designing a practice milk quality lab. First, the lab can perform cultures to screen for specific pathogens at a lower cost than full culture. For example, one could just streak a Factor plate for Staph aureus screening or a Prototheca plate for Prototheca. Second, whole herd screening can be an opportunity to provide clinic staff to collect samples on a dairy. This provides expert collection skills and additional help to the producer and can hopefully maintain parlor throughput during the collection period. Third, there are in-line devices available for purchase that allow sampling by string or group that may enhance the marketability of a contagious pathogen screening service.
Culture based treatment of subclinical mastitis (CBST) may offer another opportunity. While it is understood that treatment of subclinical mastitis caused by environmental pathogens is mostly thought to be of little economic value,\textsuperscript{11, 4} there may be cause to question this particular dogma today. The largest cost of subclinical mastitis is milk loss.\textsuperscript{11, 4} Most of this is milk that is not produced due to the effects of infection. In the case of clinical mastitis there is additional milk loss because abnormal milk is supposed to be discarded from use for human consumption as per the Pasteurized Milk Ordinance. It is not necessary to discard milk from subclinical cases however, so the cost of treating subclinical mastitis is typically much greater than treating clinical mastitis when one considers that milk from subclinical infected cows could be sold.

However, on-farm pasteurizers are extremely common on dairy farms today, and many dairies with very good to excellent milk quality usually do not have sufficient hospital milk to supply the needs of calves, so they may use regular bulk tank milk instead. Thus the cost of “discarding” milk may be zero in such cases. The second argument against treating subclinical cases is that milk production does not increase subsequent to treatment.\textsuperscript{11} While this may be true, the same may be true for clinical mastitis and since many or a majority of clinical cases may present with normal-appearing milk within 4-6 days, treated or not, the economic return of treating grade one or two clinical mastitis versus treating subclinical mastitis may be almost entirely the cost of discarded milk due to drug residue or abnormal milk. The other costs, i.e. future culling, reduced milk production, risk of infection to other cows, may not actually be different. While it is possible that all of this is conjecture on the author’s part, our practice has found significant interest by some producers in limited treatment of subclinical mastitis.

We have found several useful components of a successful CBST program. First, this practice should be farm specific. Much like selective dry cow therapy, there are likely farms that are
suited for this practice and farms that are not. Suitable farms for CBST are probably farms with low bulk tank SCC and low rates of clinical mastitis. Farms with high bulk tank SCC and/or clinical rates probably should have higher priorities than CBST, including CBT, screening for contagious pathogens or bulk tank culture, for example. Efforts to identify and treat subclinical cases may dilute the energy of the overall mastitis control program. Second, identifying cows as possible candidates for CBST is an opportunity for veterinary involvement in record analysis. When evaluating such records, our practice’s veterinarians typically eliminate cows from consideration that have chronically elevated somatic cell counts, and cows with highly variable somatic cell counts, and cows more than 200 days in milk. Cows considered for CBST are usually “new” infections, which are identified as animals with a low previous test SCC and a high current test SCC, or cows with a low test SCC on the last test of the previous lactation, and a high SCC on the first test of the current lactation. Cows identified as eligible are recommended for a California Mastitis Test screen and culture of CMT positive quarters. Third, appropriate treatment protocols for subclinical mastitis may be different than those for clinical mastitis. An argument can be made, for example for treating cows infected with Streptococcus species and not treating cows infected with coagulase negative Staph species, because Staph-infected cows are more likely to self-cure, especially first lactation cows with high first test somatic cell counts. Fourth, record analysis to identify possible SCBT candidates can have a side benefit of identifying chronically infected cows that probably should be designated as “do not treat”, meaning that they are not to be treated if a case of clinical mastitis is observed. In our practice most farmers did not have systems in the past that could adequately find such cows, and thus they typically treated many cows that were unlikely to respond. Post-test record analysis by our veterinarians has resulted in needed improvements in this regard.
Bulk tank culture (BTC) is another opportunity. It is a great tool for evaluating milking hygiene, and combined with an in-line sampler it can be used to evaluate hygiene by milking shifts. Dairy producers sometimes fail to understand that, at least for “environmental” mastitis pathogens, bulk tank culture results can change immediately in response to a change in parlor routine, while somatic cell counts may take days to weeks to change after a modification in routine. Bulk tank culture is also a valuable tool for screening for contagious pathogens, including Prototheca in our experience. We are not aware of any studies showing that Prototheca in bulk tank milk corresponds to Prototheca infected cows in the herd, but we have seen instances where identifying and culling Prototheca cows results in immediate reduction of Prototheca isolation in bulk tank milk. While Prototheca from bulk tank milk can grow on blood agar and other agars, growth is more likely on Prototheca-specific plates, in our experience. An opportunity may exist for clinics located near milk processing or collection plants to perform periodic, typically monthly, BCT for patrons of the processor, since many processors currently provide BTC results to patrons. Potential pitfalls of bulk tank culture are frequency of sampling, storage, and transport. For adequate sensitivity and specificity at least three separate milkings or separate milk pick-ups should be tested. There is sometimes reluctance by producers or milk truck drivers, in cases where the driver is responsible for sampling, to comply. Samples must be collected in a sterile manner and must be frozen immediately to keep organisms from multiplying. Samples should arrive at the lab frozen, for the same reason. Specific bulk tank culture methods used in our lab were acquired from the University of Minnesota Laboratory for Udder health, and then modified slightly.

Culture based selective dry cow therapy (SDCT) may be an emerging opportunity for an in clinic lab. SDCT has been advocated to reduce antibiotic use and reduce costs on United States dairy
farms in recent years. There are no current standards for selection of appropriate candidates
within herds however. Using quarter cultures at dry up has been proposed and evaluated as on
possible selection method.9 Dry up cultures could be performed in a practice lab.
Quantitative bedding culture can be used to measure the potential exposure of teats to pathogens
in bedding. Recently it has been demonstrated that increased bacterial counts correspond to
increased somatic cell counts10 and increased infection rates16. While a pitfall exists of a lack of
standards, a variety of standards have been proposed and can be used as a basis for
recommendations. The methods used for bedding culture in our laboratory came from the
Laboratory for Udder Health at the University Of Minnesota College Of Veterinary Medicine.a
Other veterinary diagnostic laboratories may be alternative sources for bedding culture methods.
However, we are not aware of standardized procedures for bedding culture.
Quantitative towel culture is another potential opportunity. Bacterial levels on towels have been
shown to increase infection rates15. Bottlenecks to providing clean towels exist on many dairies,
including dirty washers and driers, inadequate amounts of hot water, poorly functioning
equipment, poor washing and drying technique, and improper storage. As with bedding culture,
there are no universal standards, however in our experience clean towels will have bacteria levels
close to zero. Towel culture techniques in our laboratory are also taken from the Laboratory for
Udder Health.a
Water culture can easily be performed in a clinic laboratory. Our laboratory typically cultures
water to evaluate if pathogens are present in water that is used for udder preparation, parlor
cleaning, or milking equipment cleaning. Non-coliform gram negative organisms are often
found in water. Pseudomonas species and Serratia species are the most common isolates found
in our laboratory. Coliforms may be present, but often may be due to improper sampling.
Pseudomonas and Serratia can cause clinical mastitis. It is very common to find one or more of these organisms in stored water. Water on dairy farms is often stored in underground cisterns and above ground tanks; farms may need stored water to meet the demand for water during high demand periods of the day. When contaminated water is used for udder preparation or to clean the surface of milking equipment mastitis may result. Serratia and Pseudomonas may also be found in parlor drop hoses, plate coolers, water preheaters, and even teat dips. Non coliform gram negatives, particular Pseudomonas species, may be associated with high Plate Incubation (PI) counts in milk and may affect the market for a farm’s milk. We use a standard of zero colonies per ml of water for water samples, but again, we are not aware of any specific udder health standards for water culture. Our lab uses a procedure that is also from the Laboratory for Udder Health and the University of Minnesota.\(^a\)

Quantitative culture of calf milk and colostrum, while not technically part of a milk quality lab, are another opportunity for a laboratory and fit well with the type of procedures and expertise available in a typical lab. Colostrum and calf milk culture are relatively commonly performed in the dairy industry today; thus many producers are familiar with these tests, which might make marketing of such less difficult than in the past. McGurk\(^{12}\) has shown that excess coliforms in calf milk can cause illness, and others have shown that excessive bacteria levels in colostrum can reduce IgG absorption.\(^6\) As a result calf milk and colostrum culture can enhance the quality and effectiveness of a calf health management program, much like a milk quality lab can enhance a milk quality program. It is important to sample calf milk and colostrum at the point of feeding to estimate the actual bacterial load ingested. For both milk and colostrum it may be desirable to sample both pre and post pasteurization to evaluate pasteurizer function. In automatic milk feeding systems it may be necessary to sample at multiple points to identify areas that are
potentially contaminating the milk or milk replacer supply. In our experience, automatic feeders often do not clean well, so contamination from the feeder itself is not uncommon. Furthermore, larger automatic feeder barns may have feeders and a bulk tank connected by a continuous flow loop, where milk circulates back from the loop to the bulk tank, which introduces another potential area of contamination should the line cleaning system not work properly. One potential pitfall with colostrum culture is that it often needs to be performed at a different dilution than calf milk, because it is often produces many more bacterial colonies per ml than milk, making counting difficult. Occasionally the test may need to be repeated at a different dilution as a result. Another potential pitfall is that calf milk and colostrum quantitative standards vary and may require some thought and input to develop appropriate standards for one’s lab. Another potential pitfall is implicating pasteurizer malfunction when the real problem is contaminated raw milk or colostrum. Pasteurizers can be reasonably expected to reduce pathogen loads by 95%, but excessively dirty raw product will still result in a dirty pasteurized product. On the other hand, such experiences produce a teachable moment where the practitioner can discuss proper collection and handling of calf milk and colostrum with the farmer. Procedures used in our lab for calf milk and colostrum culture were acquired from the Laboratory for Udder Health, University of Minnesota.\textsuperscript{a}

\textit{Procedures, Methods and Operations}

For BCT, calf milk, colostrum, water, towel and bedding culture, see above.

For individual cow or quarter culture identification the most cited source of methods is the \textit{Laboratory Handbook of Bovine Mastitis}, available from the National Mastitis Council. Papers often refer to “standardized methods” as per the NMC handbook. However, there really are no standardized methods, and this may be a serious pitfall for someone considering an in house
laboratory. The Handbook does specifically discuss certain methods, such as plating samples, and performing diagnostic tests, but it does not provide a complete flow-based description of how one would proceed identifying organisms grown from a milk sample. Indeed, there are many different possible ways to get to a final identification. For example, it would be appropriate to plate milk on only a blood agar plate at first, and some laboratories do this, but our laboratory uses four plates: blood agar, TKT, Factor, and MacConkey’s, for the initial plating. We do this because we want to provide preliminary results by the next morning and using growth patterns on four plates makes this goal easier to achieve. The specific procedures for organism identification use by our laboratory are outlined by Bennett. There are a couple notable changes in procedure since that document was published however. First, methods for identification of esculin positive Streptococci and Streptococci-like organisms are significantly different. Our laboratory uses sorbitol fermentation to divide these organisms into either Lactococcus species, or other esculin positive Streptococci-like organisms, including Strep uberis, Enterococcus species, and Aerococcus species. We then differentiate into either Strep uberis or Enterococcus species using bile esculin plates but do not try to identify Aerococcus since it is relatively uncommon in bovine milk. Differentiating Strep uberis from Enterococcus species may be important since Enterococcus species are inherently resistant to cephalosporins, and cephalosporins are very commonly used to treat clinical mastitis. Furthermore we no longer use the PRY test, described in the document, because we found it to give unreliable results. A pitfall of organism identification is that there often a variety of ways to get a result, and not every procedure will give the same results every time. Indeed, there are often times when two individuals may read a certain test result differently, even if performing the same test in the same way. Thus organism identification in an in clinic lab will never produce correct results 100% of the time. It should
be noted though, that procedures with much greater specificity such as 16s RNA typing or MALDI-TOF are not necessarily 100 percent accurate either, since both require that a colony grow on an agar plate and be selected for analysis by a human, and it is possible that a different person might select a different colony, or that additional plating of the same sample could produce a colony of a different organism. A reasonable goal for an in house laboratory is to not make many mistakes that matter regarding the ultimate outcome of the case. For example identifying a colony of Staph aureus as coagulase negative Staph species might result in treatment failure, or failure to segregate or cull a cow infected with a contagious pathogen, while misidentifying a colony of Enterobacter species as E. coli may not adversely affect outcomes. Thus another pitfall of an in house lab may be failing to understand the limitations of in house diagnostics and failing to adjust recommended protocols accordingly.

The second major change to procedures as outlined by Bennett, ET. al., is the number of colonies considered to be “significant growth”. Instead of requiring three similar colonies for environmental pathogens we now require only one colony, except for coagulase negative Staph species, where we require 2 colonies. This is done to increase sensitivity as described by DoHoo⁵. A related pitfall is determining the threshold for what is a “contaminated” sample. For example if there is very significant growth of Strep uberis and one colony of E. coli and one colony of Klebsiella, does that make for a contaminated sample? The answer to this question will depend on the goals of the laboratory. For culture based mastitis treatment, it would be appropriate to report the results as Strep uberis in the above sample, because that is the most likely pathogen isolated. However, an alternative method might be to report all organisms with some measure of the degree of growth. If this method is chosen though, then treatment protocols need to be designed so farm managers and employees can easily determine the appropriate
treatment for each sample submitted. A result of four or five organisms, by itself, would not likely be helpful. The protocol used for determining contamination, and how results will be reported, need to be standardized and documented.

Another pitfall regarding individual identification is that organisms do not always produce test results as predicted even when tests are performed correctly. In some respects organisms may be seen as a continuum rather than discrete entities, so some Klebsiella isolates may have produce test results more in common with Enterobacter species than other Klebsiella, for example.

Perhaps the greatest pitfall regarding identification is that one can report results based on poor or inconsistent procedures that a client will then use to make treatment decisions. It can be tempting to look at a colony on a plate and pronounce a diagnosis where more careful consideration might find a different result based on more testing. It is this author’s opinion that poor results from a mastitis laboratory are worse than no results for this reason.

To improve quality control it is advisable to have a backup diagnostic laboratory to which one can send samples with questionable results. There are many laboratories available for this purpose. In addition, the lab may consider subscribing to a quality testing service as is offered by QMPSc where samples are sent to member labs for identification, after which the correct results are shared by the testing laboratory. Another useful quality control method is to purchase some control organisms. These are samples of live, identified organisms that can be used in the lab to periodically test ones procedures.

An opportunity to reduce errors exists by standardizing and documenting procedures. Documentation can also be in the form of a checklist where an individual marks a box or initials a form to indicate that a procedure was completed. Our laboratory uses a variety of documents
toward this end. For example we have a laboratory log located on a clipboard in the lab. The log has the following columns: Date submitted, veterinarian, client, number of samples, test requested, date set up and initials, client notified and initials, veterinarian notified and initials, billed and initials, and billing checked with initials. The goal is to reduce errors. While a sample is in the laboratory a tracking sheet is created for each sample that has columns for every test performed, along with boxes indicating that the results were checked by a veterinarian, and that the results were reported to the client and when. While much of this might seem redundant we have found that documentation has greatly reduced errors while streamlining the process at the same time.

Electronic file storage is an opportunity to reduce errors and save time. Our laboratory generates reports in Microsoft Word at the lab desk computer. Results determined from the tracking sheet are entered into the report and the report is saved in a client’s file. The client is notified by client preference, either attached to an email, as a text message, a phone call, or via file sharing. File sharing utilizing Google Drive is used to report results to the veterinarian of record for each sample. With this system the veterinarian receives an email with a view of the report. At that time the doctor can delete the file, add it to a client’s folder on Google Drive, share it with the client, or add comments and then share it with the client. This system eliminates lost paper copies and makes it very simple and fast for the veterinarian to view results.

Culture Tracker is a proprietary program that creates an interface between the farm Dairy Comp 305 file and the laboratory computer. The farmer can enter sample numbers on the farm and have them transmitted electronically to the lab. Likewise the lab can enter results and have them transmitted to the farm DC 305 file.
An in house milk quality laboratory offers the veterinary practitioner a valuable tool to enhance udder health and calf health programs. It also can be an additional source of revenue. It can help clients use antibiotics responsibly. There are a variety of pitfalls to overcome however. The most significant may be difficulty in producing quality results consistently due to the lack of truly standardized procedures.

Endnotes.

a The Laboratory for Udder Health, MVDL, University of Minnesota, St. Paul, MN, mastlab@umn.edu

b Hardy Diagnosticas, Santa Maria, CA, www.HardyDiagnostics.com

c Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine, Ithaca, NY. http://ahdc.vet.cornell.edu/sects/QMPS/Programs/proficiency.cfm. Accessed April 2019

d Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine, Ithaca, NY.


Acknowledgements

The author declares no conflict of interest.

References


13. Roberson JR. Clinical mastitis: the first eight days; *Proceedings. 43rd Annu Conf Am Assoc Bov Pract* 2010; 43:124-133.73


20. *Wisconsin Veterinary Diagnostic Laboratory Spring Newsletter*. 2019