

# Using On-Farm Mastitis Culturing

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## ■ Take Home Messages

- ▶ On-farm systems provide insight regarding pathogen identification for immediate therapeutic decisions for clinical mastitis, dry cow therapy and specific pathogen treatments.
- ▶ Both the Minnesota Bi or Triplate system and the Petrifilm system provide accurate results for clinical treatment, when used by experienced and well trained producers.
- ▶ For clinical mastitis therapy decisions, antibiotic use can be decreased by using the systems without a negative effect in bacteriologic or clinical cure.
- ▶ The economics of using on-farm systems for clinical mastitis therapy depends on the rate of no-growth and coliform infections on the farm. Herds with lower levels of Gram positive infections will benefit most because of lower antibiotic and milk withholding costs.
- ▶ Preliminary results indicate that aerobic count Petrifilm can be used on low SCC cows to accurately determine infection status at dry off and that teat sealants alone can be used in these cows to prevent new intramammary infections.
- ▶ Certain Gram positive mastitis cases can benefit from more aggressive antibiotic therapy. For example, Staph aureus cases which meet certain cow factor criteria have higher cure rates when treated for longer periods. The Petrifilm Staph Express system can be used to identify Staph aureus cases and, with cow history data, make treatment and culling decisions.
- ▶ Regardless of the system used, it is important to routinely submit samples to a diagnostic laboratory for quality control and to fully identify the mastitis pathogens for developing control programs.

## ■ Introduction

Mastitis remains the most costly infectious disease to the dairy industry and is the most frequent cause of antibiotic use on dairy farms (Erskine et al, 2003). The use of antibiotics to treat mastitis results in cost associated with milk withholding due to residue concerns. In a Wisconsin study involving 20 conventional dairy herds, approximately 80% of all antimicrobial drug use was for mastitis (Pol and Ruegg, 2007). In that study, 50% of all antimicrobial drugs were for clinical mastitis. Similarly, mastitis treatment accounts for more than half of all antibiotics used by dairy producers in Canada (Leger et al, 2003). In approximately 90% of cases, antibiotic residues in milk can be traced back to mastitis treatments (Erskine et al, 2003). There are public concerns that the use of antibiotics in agriculture may lead to antimicrobial resistance in humans. Selective pressures from antimicrobial use, mutations, or acquisition of foreign resistance determinants mediate antimicrobial resistance (Tikofsky et al, 2003). For these reasons, the judicious use of antibiotics by veterinarians and producers continues to be emphasized throughout the dairy industry.

While residue risk and antimicrobial resistance are of concern, it is important to note that as an industry charged with the humane care of the animals entrusted to our care, judicious use may not always mean reduced use. In fact, targeted use may increase the intensity of use under certain conditions, while diminishing or eliminating use in other circumstances. This paper examines three specific areas for evidence-based mastitis treatment using on farm culture. These areas include treatment decisions for clinical mastitis cases, application of the technology to make selective dry cow antibiotic treatment decisions and identification of species of pathogen for use in determination of duration of treatment.

## ■ Clinical Mastitis Treatment Decisions

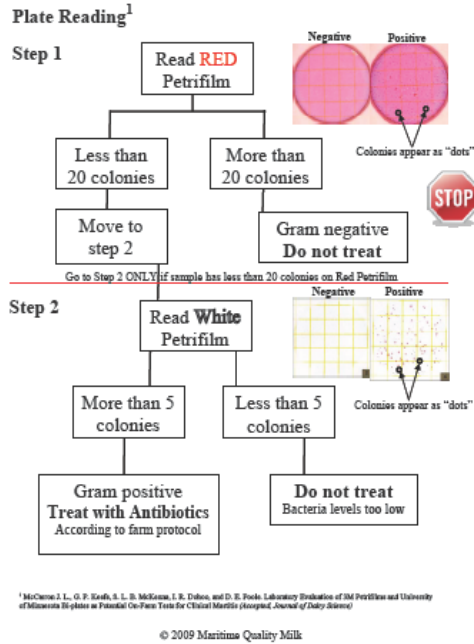
Despite improved control of contagious mastitis pathogens, the incidence of clinical mastitis remains high with rates of 7.3-36.6% per lactation (Ruegg, 2003; Olde Riekerink et al., 2008). Clinical mastitis is caused by a wide range of bacteria and appropriate therapy of clinical mastitis remains a contentious issue. When designing a treatment regime, having information on the causative organism in order to choose an antimicrobial with an appropriate spectrum of activity is important (Constable and Morin, 2003). A recent Canadian study reported that 43.9% of 3033 milk samples submitted from cows with clinical mastitis yielded no bacterial growth (Olde Riekerink et al., 2008). Additionally, early studies found no difference in clinical or bacteriological cure rates in mastitis cases caused by environmental pathogens treated with antibiotics versus those treated with oxytocin

(Guterbock et al. 1993). However, animals infected with environmental streptococci in the oxytocin-treated group had a higher rate of relapse (Van Eenennaam et al, 1995). Because this work indicated that short and longer term outcomes for animals with Gram-negative infections were not affected by antibiotic treatment, typically cases are divided into two treatment categories based on the following pathogen grouping: Gram-positive (receives antimicrobial treatment) and Gram-negative and no bacterial growth (does not receive antimicrobial treatment). This classification has been substantiated in a large study of subclinical pathogens with seven antimicrobials, where only Gram-positive pathogens had improved cure rates (Wilson et al, 1999). Using this system, Roberson (2003) estimated that antibiotics would not be justified in 50-80% of clinical mastitis cases. Historically, Gram-negative infections have been reported to have high self cure rates, however, certain strains may contain virulence factors that increase persistence (Dogan et al., 2006). With the recent approval of products with label claims against Gram-negative organisms, the appropriateness of this treatment classification needs to be closely examined. Epidemiological monitoring of herd and cow specific recurrence rates may identify infrequent situations where more aggressive treatment protocols are required.

Antimicrobial therapy should only be used for pathogen groups where there is strong evidence of benefit. By treating fewer cases with antibiotics and discarding less milk that contains residues, the cost per case of mastitis can be reduced (Godden et al., 2007). One method to target therapy is to use an on-farm culture system. Over the last 5 years, two groups in North America have been working on large clinical trials to evaluate the short and long-term implications of on-farm culture driven selective clinical mastitis therapy. The complimentary studies looked at on-farm studies in a large number of smaller Canadian herds through the Canadian Bovine Mastitis Research Network (CBMRN) and a smaller number of larger Great Lakes Region dairy herds.

## **Canadian Laboratory Study**

The CBMRN study was phased to first directly compare previously described tools that might be adapted for on-farm use in the Canadian industry (McCarron et al., 2009a). Two systems were evaluated: Petrifilm (3M Canada, London ON) and University of Minnesota Biplates (U of Minnesota, St. Paul MN). For the Petrifilm-based system, two media are required to classify cases as Gram-positive, Gram-negative or no significant bacterial growth: aerobic count (AC) and coliform count (CC). One hundred microliters in 1:10 dilution of each sample was plated and incubated at 35°C for 24 hours. Using the treatment algorithm from Figure 1 (see [www.milkquality.ca](http://www.milkquality.ca) for details) the sensitivity (proportion of true positive identified) and negative predictive value (proportion of test negatives that were true negatives) of the system was 93.8% and 89.7%, respectively (McCarron et al., 2009a).



**Figure 1. Plate reading algorithm for Petrifilm on-farm cultures**

The Minnesota Bi-plate contains a proprietary Factor medium that is selective for Gram-positive bacteria on one half plate and the other half contains MacConkey medium for the identification of Gram-negative bacteria (Laboratory for Udder Health, 2000). The sensitivity and negative predictive value of this system was 97.9% and 96.4%, respectively (McCarron et al, 2009a).

In addition to the test performance characteristics, the user operator features were examined. The shelf-life of the Petrifilm system, validated in the laboratory, is at least 1 year, whereas the shelf-life of the Biplate system is 6 weeks. Based on the relative equivalencies of the systems and these stability properties, which may be particularly important on small Canadian farms, the Canadian study proceeded to the on-farm clinical trial with the Petrifilm system.

## Canadian Field Study

In the second phase of the study, 54 Canadian dairy farms from 7 provinces were enrolled in a clinical trial to evaluate the utility of an on-farm culture system and selective intramammary therapy on clinical mastitis cases. Cows with abnormal milk only (severity score 1) or inflamed udders and abnormal

milk (severity score 2) were randomly allocated to either an on-farm culture group (selectively treated) or an antibiotic treated control group. Cows exhibiting systemic signs of illness (severity score 3), specifically rectal temperatures in excess of 39.5°C, were not included in this study. Milk samples were collected at the onset of mastitis (M1) and submitted for standard bacteriologic culture. A second milk sample was inoculated (1ml of 1:10 diluted milk) on each of the CC and AC Petrifilms. Incubation was performed at 35°C for 22-24 hours in a Turbofan Hovabator (GQF Manufacturing, Savannah GA). Petrifilms were read by the dairy producer using the treatment decision algorithm (Figure 1). Cases with significant Gram-positive growth and cases with no significant bacterial growth or Coliform infections were classified as treatment and no treatment cases, respectively.

In addition to follow up milk samples, clinical progression data were collected including cow demographic information, dates of onset of clinical mastitis, the date that milk returned to normal appearance and the date milk returned to the bulk tank for sale. On-farm culture results were recorded as well as all treatments instituted in each case. All cows contributing cases were followed up for a minimum of 4 months to assess relapse of clinical signs. Clinical cure was assessed based on milk returning to normal appearance as recorded by the producer. Cases where the milk failed to return to normal or required additional antibiotic treatments beyond the constraints of the assigned treatment protocol were recorded as failures. Bacteriological cure rates were determined by clearance of the causative organism 14-21 and 28-35 days post event.

In total 997 clinical cases were enrolled from which 621 from 48 farms met all criteria and had complete records (all necessary samples and recorded information) available. From the M1 samples (time of incident) 55% were Gram positive, 11% were Coliforms and 24% had no significant growth on standard microbiology in CBMRN laboratories. Ten percent of the farmer collected samples were contaminated or contained yeasts or fungi. Using the on-farm system, 60% of samples were identified as Gram positive, leading to a reduction in antibiotic treatment in all cases by 40%. Test characteristics on-farm were highly variable based on the number of cases contributed by each farm. Sensitivity and negative predictive value deteriorated linearly from 87.5% and 90.3%, respectively, for herds averaging 1.5 cases per month (18 cases over the study period) to 65.9% and 62.2% when all herds were considered. Based on these data, the accuracy of on-farm culture appears to be highly dependent on farmer training and experience.

Cases were deemed to not have cured if additional antibiotics were used, the cow became systemically ill, the cow was dried off or culled due to the case or the milk did not return to normal. Bacteriologic cure was defined as not having the organism present on the M1 sample or either the M2 (14-21 days

post case) or M3 (28-35 days post case) samples, whereas clinical cure was a return to visibly normal milk. There was no difference in the bacteriological cure rates of the control (63%) and on farm culture groups (56%) and there was no difference in the overall clinical cure rate (78 vs. 77%) or overall days to clinical cure. Cases that were misdiagnosed (not treated when they should have been or treated when it was not required) had longer days to clinical cure than appropriately diagnosed cases. Despite problems on some farms with misclassification, because the untreated group did not have to wait out withhold times after the milk returned to normal appearance and there was reduced drug costs, there was a net economic benefit for the producer and a return on investment of approximately 2.5:1 (preliminary analysis).

## Great Lakes Study

The Great Lakes Region study lead by Dr. Sandra Godden involved 8 herds (2 in Minnesota, 5 in Wisconsin and 1 in Ontario) with herds ranging in size from 150 to 1800 cows (Lago, 2009). Similar to the CBMRN study, animals were randomly assigned to positive control (immediate treatment with Cephapirin Sodium) or culture based treatment using the Biplate. In total 449 quarters were enrolled in the study. In that study 44% of cases in the culture group were treated with Cephapirin (Gram-positive or mixed), whereas 66% were either Gram-negative or had no bacterial growth.

Outcomes from the study were examined as short and long term effects of the selective therapy program. For short-term outcomes, there was a tendency for a reduction in the number of days out of the tank for cows assigned to the culture-based group (5.2 vs. 5.9 days). The reduction in days out of the tank for the group not treated with antibiotics was partially offset because of the longer days out of the tank for the culture and subsequently treated group (as in the Canadian study), perhaps because treatment of these Gram-positive infections was delayed for 24 hours. Quarters that were in the on-farm culture group had lower risk of extended or secondary treatments for the case. There was no difference in days to clinical cure or the bacteriologic cure rate for the two groups. Similarly there were no differences between the selectively treated group (based on on-farm culture) and the blanket treated group for presence of infection, clinical mastitis recurrence and risk of removal after the mastitis case (Lago, 2009).

For longer-term outcomes from this Great Lakes study there were no significant differences between the cows treated selectively using the on-farm culture system and the blanket treated cows for days to subsequent mastitis event and risk of and days to culling. The linear score somatic cell count and lactational milk production were not different between the groups.

Development of farm treatment protocols should be done at the herd level in consultation with the herd veterinarian. Herds that have higher no-growth or

coliform mastitis rates will have more economic advantage by using on-farm culture versus herds with high Gram positive infection rates. The treatment protocols in each of these studies dictated no antibiotic therapy for cows with no significant bacterial growth or mild (no systemic signs) coliform cases. Monitoring for the emergence of chronic stains should be done at the farm level. If chronic strains emerge, a farm treatment protocol that includes targeted treatment of Gram-negative infections with appropriate products should be considered.

## ■ Selective Dry Cow Therapy

Since the development of the Five Point Mastitis Control Plan during the 1960s, it has become common practice to treat all cows at dry-off with an intramammary antibiotic. The purpose of dry cow therapy (DCT) is to clear up any existing infections and to prevent new infections from being acquired during the dry period. Dry cow intramammary antibiotics decrease the risk of new infections for a short period after administration, but their activity diminishes over time and they are not protective in the late dry period, just before calving (Oliver et al., 1990). Internal teat sealants act as a physical barrier in the teat canal and prevent bacteria from entering. While intramammary antibiotics lose their efficacy over the length of the dry period, internal teat sealers have been shown to reduce new mastitis cases throughout the entire dry period. The use of internal teat sealers prevented intramammary infections (IMI) at calving, reduces clinical mastitis during the dry period, and results in less clinical mastitis in the first 100 days, when compared to no treatment (Berry and Hillerton, 2002). Studies at the Atlantic Veterinary College have shown that teat sealants are just as effective at preventing new IMI during the dry period as DCT in cows that do not have infections at dry-off (Sanford et al., 2006).

The use of selective dry cow therapy has been advocated (and successfully implemented) in some countries (Osterås et al., 1999). While internal teat sealers are a practical alternative to DCT for the prevention of new IMI, they have no antimicrobial capacity and should not be used alone in animals with preexisting infections. If producers were able to determine which cows had an IMI at the time of dry-off and which cows did not, they could make selective dry cow treatment decisions (sealant only vs. antibiotic) for each individual animal. This in turn would reduce the costs associated with dry cow treatment (for those using antibiotic and sealants) as well as address concerns with over-use of antibiotics and antimicrobial resistance without compromising the long-term health of the cow.

A preliminary study to assess the ability of on-farm culture systems to detect cow infection at dry off was conducted at the Atlantic Veterinary College (McLaughlin et al, 2010). In this laboratory study, the aerobic count Petrifilm

test correctly identified infection status, with 100% sensitivity and 100% negative predictive value at 5, 10 and 20 colony count cut-off values . Using the same colony counts the specificity was 70, 82, and 84%, respectively. Based on these data, a selective dry cow field study began in July 2009 on 16 farms located in Quebec (in conjunction with Jean-Philippe Roy at the U. of Montreal) and Prince Edward Island. In this study, eligible cows (low SCC) were randomly assigned to a positive control group (receiving dry cow antibiotic and teat sealant) or an on-farm culture group (Petrifilm). For those in the on-farm culture group, culture positive cows (5+ colonies on Petrifilm) received both internal teat sealant and antibiotic dry cow therapy, while those culture negative received teat sealant only. Full data is available for cows enrolled in the first 6 months of the project (n= 336). In the study group (n= 164), 86 (52.4%) cows cultured negative on-farm and received an internal teat sealant as a sole treatment. When read by the producer, the Petrifilm had a sensitivity of 92.6% and a specificity of 73.1%. The risk of new IMI over the dry period was not different between the positive control and on-farm culture groups at 14.4 and 17.8%, respectively.

## ■ Pathogen Species Identification

The primary focus of on-farm diagnostic systems is to make timely therapeutic decisions. For epidemiologic monitoring of the main udder health pathogens in the herd, as well as quality control for on-farm systems, it is important that producers and their veterinarians routinely submit duplicate milk samples to accredited diagnostic laboratories. One organism of interest for therapeutic decision making is *Staphylococcus aureus*. When a diagnosis of Staph aureus is made the producer has 3 options: 1. withhold lactational treatment with the consideration of culling or dry cow therapy at a later time; 2. treatment with the conventional farm protocol (typically 2-3 days) or 3. treatment with prolonged therapy. Response to therapy varies widely based on cow and pathogen factors. Many of these factors (cow age, number of quarters infected, extent of SCC elevation and chronicity of SCC elevation) are available on-farm to make treatment decisions after diagnosis. There is substantial evidence that if the decision to treat is made, longer duration therapy yields improved cure-rates over conventional therapy (Barkema et al., 2006).

Godden et al. (2007) evaluated the University of Minnesota Tri-plate culture system for the ability to differentiate growth from no growth, Gram-positive from Gram-negative and growth of Staph vs. Strep species. They found the system to be accurate and attained a high level of agreement (kappa values between 0.80 and 0.93) when used for those three purposes. However, when users tried to differentiate pathogen groups further or to identify specific pathogens (Staph aureus vs. CNS) much poorer agreement was observed. In a second study, the Tri-plate was highly sensitive (97.9%) with moderate



specificity (81.8%) when used by a laboratory technician experienced in hemolytic pattern recognition, to diagnose the presence of *Staph aureus* (McCarron et al., 2009b). However, when read by four inexperienced readers, the sensitivity was much lower (ranging from 43.2% to 59.1%).

The Petrifilm Staph Express (STX) plate is designed to provide rapid results for the diagnosis of *Staph aureus* after  $24 \pm 2$  h of incubation. The manufacturer's interpretive criteria suggest that the appearance of red-violet colonies on the initial incubation is presumptive evidence for the diagnosis of *Staph aureus*. Results from Silva et al. (2005) did not support this recommendation and found it necessary to use the Staph Express Disk, which measures DNase activity, for confirmation, even when red-violet colonies were the only type present. The study of McCarron et al. (2009b), agreed with the findings of Silva et al., that to improve specificity for *Staph aureus* required the use of the Staph Express Disk. Overall, when using the confirmatory disc, the sensitivity and specificity of the Staph Express system was 92.1% and 93.1%, respectively. Accuracy in the diagnosis of *Staph aureus* is important because segregation, culling and therapeutic decisions are being made based on these data. The high sensitivity and specificity values from both the McCarron and Silva studies indicates that using the STX Petrifilm resulted in very few false negative or false positive classifications. Each study recommended the use of the confirmatory disk. The application of the STX disk did not improve the sensitivity of the test as the manufacturer suggests, rather the specificity of the test was greatly improved. Regardless of the method of on-farm culture system employed it is recommended that periodic assessment of accuracy of on-farm methods by submission of duplicate samples to a microbiology laboratory be carried out.

## ■ Acknowledgements

This paper summarizes 3 research focus areas for the authors. The clinical mastitis treatment decisions and the mastitis pathogen speciation projects were sponsored by the Canadian Bovine Mastitis Research Network, which is financed by the Natural Science and Engineering Research Council, Alberta Milk, Dairy Farmers of New Brunswick, Nova Scotia, Ontario and Prince Edward Island, Novalait Inc., Dairy Farmers of Canada, Canadian Dairy Network, Agriculture and Agri-Food Canada, Public Health Agency of Canada, Technology PEI Inc., Université de Montréal and the University of Prince Edward Island. The selective dry cow treatment project was a collaboration with the Université de Montréal (Dr. Jean-Philippe Roy) and was supported by Maritime Quality Milk and Novalait. Field and laboratory technical support was provided by Maritime Quality Milk at the Atlantic Veterinary College. Materials were provided by Wyeth Animal Health, Pfizer Animal Health and 3M Canada.

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