On-Farm Mastitis Diagnosis

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Introduction

There are inexpensive and rapid bacteriologic tests available that allow veterinarians as well as dairy producers to make real-time decisions to better manage udder health in their herds. The commercial on-farm culture system (OFCS) that will be discussed include Petrifilm™ plates (3M Microbiology) and Minnesota Easy Culture System. Petrifilm™ plates are ready-to-use culture media that are used primarily in the food industry. The University of Minnesota has developed the Easy Culture System, which offers two types of selective media culture systems; the Bi-plate and Tri-plate. These OFCS enable us to rapidly diagnose and implement a targeted treatment regime (for clinical mastitis) or control program (for contagious mastitis).

Clinical mastitis is the primary reason for antibiotic use in dairy cow (Guterbock, 1994; Erskine et al., 2003). Ideally, treatment should be based upon culture results. However, issues associated with collection, shipping and handling of milk samples, cost, and the lag-time in reporting results have made some producers reluctant to have milk bacteriology performed.

Studies have shown that in most cases of mild clinical mastitis, treatment can be delayed for 1 day (while waiting for the culture results) with minimal adverse effects (Silva et al., 2004). Using milk culture and targeted therapy has the potential for less antibiotic use and milk loss. Reason being that approximately 10-40% of clinical cases yield no-growth and do not require antibiotic therapy.

Mastitis control programs

The isolation and identification of mastitis-causing pathogens is the fundamental aspect to milk quality and udder health control programs (Ruegg, 2005). There has been a variety of culture programs suggested and implemented for dairy herds (Leslie, 1994; Gonzalez and Wilson, 2002; Kelton and Godkin, 2000). These programs include 1- periodic culture of all milk cows; 2- strategic culturing of new additions and all cows and heifers at dry-off and/or freshening; 3- culturing all clinical mastitis cases; 4- periodic culture of bulk tank milk samples. Which program or combination of programs used in a specific herd will depend on the goals and udder health objectives of that farm.

Culturing milk of clinical mastitis cases should be an important component of environmental control programs. Contagious pathogens may also be identified through this process (Kelton and Godkin, 2000). These samples can be used to identify the most common mastitis-causing pathogens in the herd (Roberson, 2003). These samples are not used to guide therapy decisions for the current case of clinical mastitis but used to help base future case therapy decisions because it is difficult to receive results from the diagnostic laboratory quickly enough to implement a therapy decision (Sears and McCarthy, 2003).
For contagious mastitis control program success, early detection of infected cows is of primordial importance (Barkema et al., 2006). *Staphylococcus aureus* positive animals need to be identified and dealt in such a way to reduce spread of the pathogen within the herd (Zadoks et al., 2002). Primiparous cows that have an IMI due to *S. aureus* at calving can also act as reservoirs of this contagious pathogen to herd mates as well as increase the prevalence rate in the herd (Roberson et al., 1994).

**Methods to diagnose Mastitis**

There are many different methods to diagnose the cause of mastitis in dairy cows. Diagnostic methods for determining mastitis include SCC and microbiological analysis (Kirk et al., 1994, Ruegg, 2005). Somatic cell count is an indirect method that detects the presence of inflammatory cells in the milk. Somatic cell counts are used to identify cows that have potential to have an IMI (NMC, 1999). Using a threshold of 200,000 cells/ml, the Se and Sp of detecting any pathogen responsible for an IMI is only 73% and 86% respectively (Dohoo and Leslie, 1991). This test is usually performed monthly by Dairy Herd Improvement (DHI) program; Valacta in Quebec. Another indirect test is the California Mastitis Test (CMT). The CMT is a surrogate measure of SCC in milk, and is a rapid cow-side test for subclinical mastitis.

**Bacteriologic Diagnosis**

There are many bacteriologic methods used to determine the type of pathogen that causes an IMI or clinical mastitis; standard bacteriology of milk, the Minnesota Bi-plate and Tri-plate system, and the Petrifilm.

Standard bacteriology of bovine milk is currently the gold standard for the identification of pathogens in milk (Ruegg, 2005; Sears and McCarthy 2003). This method consists of inoculating 0.01 mL of milk onto a blood agar plate. Examination of the blood agar plates is performed 18-24 hrs after inoculation. The final diagnosis is normally obtained at 48 to 72 hrs. From a bacteriology point of view, the organisms that cause mastitis can be divided into five groups: gram-positive cocci, gram-negative bacteria (coliforms), *Corynebacterium, Mycoplasma*, and others (*Nocardia, Prototheca*, and yeast) (Sears and McCarthy 2003). The majority of these pathogens will grow on blood agar, except for *Mycoplasma* (Sears and McCarthy 2003).

The major advantage to bacteriologic culture is the complete identification of bacteria from the milk sample, including the less common pathogens (Prototheca, Norcardia, and yeast). *Mycoplasma* can also be identified using a modified Hayflick medium (Gonzalez and Wilson, 2003). The disadvantage to bacteriologic culture that often leads to the underutilization of this method is the lag-time for results (Ruegg, 2005; Sargeant et al., 2001). Often there is a 4-5 day delay from sample submission to receiving results, therefore making targeted treatment decisions for clinical mastitis based on bacteriology difficult. Other disadvantages to bacteriologic culture, which also apply to other culture based methods, include failure to recover pathogens, and contaminated samples.

**Minnesota Easy Culture System II: Bi-plates & Tri-plates**

The University of Minnesota has developed the Easy Culture System, which offers two types of selective media culture systems; the Bi-plate and Tri-plate. The Bi-plate system is intended to identify a quarter as infected with GP or GN organisms. The culture media consists of half blood agar with 1% esculine and half MacConkey media (Godden et al.,
The Tri-plates give more complete identification by having an additional media selective media (with TKT) that allows just streptococci growth (Sears and McCarthy, 2003). The test is performed by swabbing the milk sample onto the plate, incubate and read at 24 hours. If there is no growth observed, plates are rechecked at 48 hours.

The Bi-plates have been evaluated for their ability to differentiate between GP and GN pathogens in mild to moderate cases of clinical mastitis (CM) and in fresh cow samples (McCarron et al., 2009; Lago et al., 2006; Hochhalter et al, 2006). The sensitivity and specificity of the Bi-plate was 97.9% and 68.6% respectively for mastitis cases (McCarron et al., 2006). In the study of Lago et al., the Se and Sp were 83% and 90% for CM and 88% and 70% in fresh cow samples, respectively, as compared to bacteriologic culture. The Bi-plates were also evaluated in herds using the on-farm culturing system (OFCS). There were minimal adverse effects, in those cows treated after waiting 24 hours for OFCS results after diagnosis of mild to moderate mastitis, as compared to cows treated immediately (Wagner et al., 2007).

The test characteristics of the Tri-plates were evaluated by Jones et al. (2006) for their ability to differentiate between gram positive versus gram negative growth and CNS versus Streptococcus spp. as compared to standard bacteriology. In 101 quarter samples of mild to moderate clinical mastitis cases the sensitivity and specificity to determine GP growth versus GN growth was 94.1% and 100%, respectively. When the Tri-plate was evaluated on its ability to differentiate CNS versus streptococci spp., the Se and Sp was 78% and 67%, respectively. In 210 quarter samples taking at calving, the test characteristics for the same series of comparisons was 95.7% and 73.9%, and 83% and 74%, respectively. These values are similar to those of the Bi-plate and allow for more complete microbiological analysis for streptococci spp. A more recent study by McCarron et al (2009b), evaluated the Tri-plates for their ability to isolate *S. aureus* and Streptococcus species in CM. The Tri-plate had a Se and Sp of 97.9% and 81.8% for detecting *S. aureus* when plates were read by a laboratory technician. The Se decreased and ranged from 43.2% to 59.1% when read by four different readers with limited laboratory training. For Streptococcus species determination, the Se and Sp were 92.6% and 89.5% respectively.

The Minnesota Easy culture system allows for relatively simple microbiological analysis to be performed on-farm in 24 to 48 hours. However, it is important to note that this system does not intend to replace commercial laboratories nor does it identify all the organisms present (such as *Mycoplasma*, yeast etc.) (Minnesota Easy Culture System II Handbook. 2000). The Bi-plates and Tri-plates have a shelf-life of approximately six weeks (under ideal handling conditions).

**Petrifilm™**

Petrifilm™ plates (3M Microbiology) are sample-ready selective culture media that are used to the rapid identification and numeration of bacteria. They are currently widely used in the food industry (Tassinari et al., 2005; Ingham et al., 2003). The Petrifilm plates that have potential for use as diagnostic tests in the dairy industry are the the Petrifilm™ Staph Express count plate, the Coliform count plates, and the Petrifilm Aerobic count plates. The Petrifilm culture system requires 1 mL of milk be added to the Petrifilm plate, incubated at 37°C and results are available in 22-24 hours.

**Staph Express**

The Petrifilm™ Staph Express count plate (STX) contains chromogenic, modified Baird-Parker media that is selective and differential for Staphylococci. After 22-24 hours, a
positive Petrifilm will present with red-violet colonies. The confirmation of *S. aureus* is performed by using a Staph Express Disk that contains deoxyribonuclease and a dye that reacts to produce a pink zone around the *S. aureus* colonies. The disk will also occasionally react with *Staphylococcus hyicus* and *Staphylococcus intermedius*.

The Petrifilm STX was evaluated for detection of *S. aureus* in bovine milk (Silva et al., 2005). The gold standard used to comparison was the isolation of *S. aureus* from any of the four microbiological techniques of standard culture, centrifugation, incubation, and the Petrifilm. The sensitivity for *S. aureus* detection was 87.5%, which was significantly higher than standard microbiological techniques (65%). The specificity of the STX was evaluated using different interpretation parameters. The specificity of the STX when a distinct pink zone was present was 98.5%. Using weak pink zones to diagnose *S. aureus*, the specificity was only 77.6% which would result in a high false positive rate. They also concluded that the interpretation of the Petrifilm was highly dependant on the reader’s ability to identify colony colour and distinct pink zones after application of the Staph Express disk plates.

Wallace et al (2008) evaluated the ability of the STX to detect *S. aureus* in milk samples from cows with high somatic cell count (n=300), mastitis (n=514) and post-partum (n=1204). The Se and Sp for each of these categories was 85.1%, 96%; 66.7%, 97.8%; and 74.2%, 97.8%, respectively. They found that the test characteristics of the STX were the highest after using diluted (1:10) milk samples.

Rapid Coliform Count plates

Rapid Coliform Count plates (RCC) are used for the rapid detection of coliforms. It is currently the fastest approved coliform test for the enumeration of coliforms in food (3M, 2001). This Petrifilm can detect high levels of coliform contamination (>1000 plate) as early as six hours during incubation. Total confirmed coliform counts can be available in 14 hours as indicated by a color change around the potential colonies. At 24 hours, coliform colonies will appear red and have gas bubbles. The RCC uses a modified violet red bile lactose nutrient base.

Wallace et al. (2008) evaluated the ability of the RCC to detect coliforms at 12 and 24 hours. The Se and Sp of the color change indicator at 6-12 hrs was 61.9% and 95.5%, respectively. At 24 hours the Sp and Sp improved to 76.4% and 96.4%, respectively.

The drawback to the Petrifilm RCC plates is the cost. The RCC plates are approximately over double the cost ($2.60 versus $1.00) of the Petrifilm *E. coli/Coliform count plates that have only the 24 hour results. Regardless of cost, the RCC may be very useful for veterinary practitioners as well as dairy producers, when faced with a case of acute clinical mastitis. Portable incubators and immediate plating of milk samples on farm will allow for the most rapid diagnosis possible. Preliminary results could be reported as soon as 8-12 hours so that appropriate therapy could be implemented.

Petrifilm *E. coli/Coliform Count plate*
Petrifilm E.coli/Coliform Count plates (CC) are used for the detection of coliforms. The difference between this plate and the RCC is that the CC does not have a color change indicator at 14 hours. Final results are only available in 24 hours.

**Aerobic Count plate**

The nutrient base used for the aerobic count plate (AC) is a modified standard method. All colonies that grow on the AC appear red. The AC is currently approved as the AOAC official methods of analysis for raw/pasteurized milk, dairy products, and foods.

The main advantages of the Petrifilm culture system is the results can be confirmed in 24-26 hrs and it requires less labor and expertise. The Petrifilm plates also have a long shelf-life of 12 to 18 months as compared to other culture media such as Bi-plates and Tri-plates (approximately 6 weeks). In small (40-200 cow) Canadian dairy herds, the logistics of maintaining culture media current is an issue. Therefore, the Petrifilm media may fill this need.

**On-Farm Culture Systems**

By knowing the causative organism on-farm, targeted therapy can be implemented. Culture results can provide valuable information for the implementation of a targeted treatment regime for clinical or subclinical mastitis. Blindly treating all cases of clinical mastitis is costly and inappropriate (Roberson, 2003; McCarron and Keefe, 2008). Ten to 50% of milk cultures from cases of clinical and subclinical mastitis yield no growth (Makovec and Ruegg, 2003; Bartlett et al., 1992; Erskine, 1991). Milk samples with positive growth may be divided into categories such as GP, GN and other (yeast, algae, etc.). Antibiotic treatment of most mastitis caused by GP bacteria has been shown to be effective and profitable (Cattel et al., 2001, Morin et al., 1998, Roberson, 2003). Of these GP organisms, not all should be treated with antibiotics such as chronic *S. aureus* (Barkema et al., 2006). A small percentage of clinical cases are also caused by less common pathogens that are refractory to antibiotic therapy (yeast and algae).

The effects of using OFCS to guide treatment decisions for clinical mastitis was recently evaluated (Lago et al., 2006). Treatment groups consisted of either immediate treatment of all mild to moderate clinical mastitis cases with an intramammary (IMM) antibiotic or waiting for culture results before treatment. The cultures were performed using the Minnesota Easy Culture System II – Bi-plate. Using the OFCS, quarters with GP growth were treated with IMM antibiotics, while GN and no growth did not received IMM antibiotics. The culture based group had a significant reduction in antibiotic use with only 43% of clinical mastitis cases receiving IMM antibiotics. When bacteriological cure rates were evaluated there was no significant difference between the treatment groups. The effects on cow health and the cost-benefit of adopting an OFCS were not yet evaluated.

Another study performed in a laboratory setting evaluated the test characteristics of two potential OFCS; the Minnesota Easy Culture System II – Bi-plate (University of Minnesota, St.Paul, MN) and the Petrifilm™ (3M Microbiology, London, ON) (McCarron et al., 2009). The gold standard for comparison was standard bacteriologic culture. The Bi-plate was evaluated for the presence of GP, GN or no growth (NG). For the Petrifilm, two plates were used to detect the presence of GP, GN or NG; the Total Aerobic Count (AC) and the Coliform Count (CC). In this targeted treatment protocol, the tests were evaluated on their ability to differentiate appropriate treatment groups. The Bi-plate had a sensitivity of 97.9% to
correctly identify GP growth and the proportion of clinical cases that would need to be treated with antibiotics was 67.9%. With the Petrifilm system using a cut-point of more than 5 colonies on the AC and more than 20 colonies of the CC, the sensitivity to correctly identify GP growth was 93.8% and the proportion of clinical cases that would need to be treated with antibiotics was 66.8%.

A retrospective cohort study surveyed dairy producers that used an OFCS and evaluated the impact of the OFCS for bacteriologic culture from cows with low-grade mastitis (Neeser et al., 2006). Ninety five percent of respondents stated that they used an antimicrobial based on the type of bacteria cultured. Farms that used on-farm culture systems and treatment protocols had a significant reduction in the rates of antimicrobial use.

The use of the Petrifilm in an on-farm culture and treatment protocol was evaluated by Silva et al., (2004) on a 600-cow commercial dairy in Wisconsin. Their objective was to use Petrifilm™ microbiological products in a protocol to appropriately use antibiotics to treat clinical mastitis caused by GP pathogens. The 3M microbiological products used in the protocol were the Staph Express, Coliform Count, and Aerobic culture media. Milk samples were collected from clinical cases of mastitis. One sample was frozen for laboratory analysis, and the second sample was used to inoculate three separate Petrifilm plates (STX, coliform, and AC). Plates were read 24 hours later and treatments were applied according to protocol. Intramammary antibiotic treatment was indicated for *S. aureus* positive cases for a new infection (first case, single quarter, and lactation 1 or 2), and for probable streptococci infections (if the AC plate was positive and STX negative). Coliform positive and no growth cases received no IMM treatment. When the 3M culture protocol was used, there was a significant reduction in days out of the tank, number of IMM tubes used per case of mastitis, and cases receiving IMM tubes. In addition, when the costs of antibiotic tubes and milk discard were considered, the cost per case of mastitis when the protocol with the Petrifilms was implemented was 90$ per case as compared to cases without a protocol at 264$ per case.

**Ormstown Veterinary Hospital Milk Laboratory**

At the Ormstown Veterinary Hospital (OVH), milk culturing started in January 2008. Currently there are two technicians and myself in charge of the milk lab. We analyse approximately 50-180 cultures per month. We have a rapid turn-around time for results at 24 to 48 hrs hours. All clients are called, faxed, emailed or texted with results 7 days a week (the information also gets forwarded to the veterinarian in charge of that herd). As well, I have a milk laboratory at my home dairy. This serves as a satellite lab for milk culture analysis during weekends and holidays and for neighbouring dairy producers to drop-off samples. We use portable incubators (the retired Hymast incubators) to transport samples between labs. Having a rapid-turn around for results allows for a value-added service. When clients are contacted, results are given, treatment and preventative protocols are then discussed.

The culture media used at the OVH are Petrifilms and Tri-plates. Milk samples from post-partum, and high SCC cows, the STX is used. Clinical mastitis the combination of STX and CC or Tri-Plate are used. The decision to use Tri-plates or Petrifilms for CM depends on the pathogen profile and treatment protocol of that farm. We also use the Petrifilm STX, CC, and AC plates to trouble-shoot hygiene issues related to calves (colostrum, milk, bottle, pails, etc).

With the Petrifilm system the milk samples are diluted 1:10 before incubation. Samples destined for *S. aureus* analysis are frozen then thawed before plating, while milk for the CC is
plated fresh (and diluted). STX plates with positive growth will have a Staph Express disk inserted to differentiate *S. aureus* colonies. When there are too many colonies to enumerate, the milk sample is diluted further. The costs of the STX, Staph Express Disk, CC, and AC plates are approximately $2.34, $1.53, $1.00, and $0.91, respectively. For further information about the 3M Microbiology products contact 3M Canada at 888-364-3577 (www.3m.com).

The Tri-plates are also easy to use. The media is inoculated by dipping a cotton-tipped swab into the milk sample, and inoculating each section of the plate, re-dipping between each section. Plates are incubated at 35°C for 24 hours. If there is no growth after 24 hours, plates are re-evaluated after 48 hours. The Tri-plates can be ordered through the Minnesota Veterinary Diagnostic Laboratory, mastlab@umn.edu, (612-624-8787). The cost of the Tri-plates and Bi-plates are approximately $2.80 and $1.75 USD plus the shipping costs.

Setting up a laboratory on farm or in clinic is simple and does not require a large initial investment. The basic material requirements are an incubator, a fridge and freezer, sterile water, syringes, cotton-tipped applicators and a clean work area.

Incubators can range in cost from $85 to $600. We use a Hovabator type incubator. This can be sourced through at Ranch Cunicol, St-Hyacinthe, Quebec (approx. $85. Phone number: 450-799-5170) or NASCO farm supply. The portable Hymast incubator is available for approximately $100 (Nelson Jameson, 800-826-8302). There is also a laboratory incubator available at 3M Canada for $557.

**Conclusion**

Are OFCS a good idea for your clinic or herds? It will depend on how you can answer the following questions:
1. Do you have different protocols for different types of infections?
2. Do you have a reliable person who has time to perform the cultures?
3. Do you have sufficient number of samples (calving/high SCC/mastitis)?
4. Are the producers and veterinarians ready to get involved with the OFCS?

If you want to use an OFCS, what type of media should you use? It depends on 1-Number of cases of subclinical and clinical mastitis (half-life of the media). 2- Prevalence of different bacteria (*S. aureus* vs streps vs coliforms). 3- The objectives in udder health (clinical mastitis vs subclinical: control *S. aureus*).

Milk cultures are a necessary part of mastitis control programs. Rapid bacteriologic tests allows for a more responsible and economic use of antibiotics. The Petrifilm, Bi & Tri-Plates are useful diagnostic test for OFCS. However, it is important to note that these systems do not replace commercial laboratories as they can not identify all pathogens present (Mycoplasma, yeast, etc.).

At the OVH, the milk laboratory and our rapid culture media are an integral component of our practice. The next step for the OVH, is to promote the use of OFCS labs in more of our herds, and combine this with herd-specific mastitis control programs.
References


