Mastitis Microbiology Simplified

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Three media and three simple laboratory techniques make mastitis microbiology a powerful tool in the hands of the practitioner. Such media and techniques are used at the Herd Milk Quality Laboratory a teaching, research, and service function of Ohio State's Depart-ment of Veterinary Preventive Medicine) and can be readily adopted in a private practice. The basic concept of the laboratory is that the determination of whether a mastitis pathogen is contagious (eg. *Streptococ-cus agalactiae* or coagulase-positive *Staphylococcus* sp.) or environmental (eg. coliform or non-agalactiae Streptococcus sp.) is fundamental to making intelligent herd milk quality management decisions.

Currently, one of two formats is selected (for initial herd culture) based upon mastitis monitoring indicators such as bulk tank somatic cell counts (BTSCC), individual cow somatic cell counts (SCC), Wisconsin Mastitis Test (WMT), or California Mastitis Test (CMT).

Format 1: Herds with a high BTSCC (>500,000) or WMT (>15). The problem in these herds is often subclinical; therefore, bacterial culture of clinical cases alone can be misleading. Furthermore, these herds often (but not always) contain a relatively high prevalence of intra-mammary infections caused by contagious bacteria. Also, contagious pathogens have a relatively long duration of infection; therefore, 1 of 2 sampling strategies is suggested:

a. Individual high SCC/CMT cows. Ideally, bacterial cultures would include those cows which have exhibited an elevated SCC/CMT for several consecutive months.
b. Random sampling of cows stratified by age and stage of lactation to be representative of the total herd.

In both sampling strategies, it is suggested that approximated 20 cows or 10% of the herd (whichever is greater) be sampled. Because of the high numbers of contagious organisms shed in milk, we suggest that composite quarter samples be taken initially.

Format 2. Herds with clinical mastitis cases but a BTSCC (<300,000) or WMT (<10). These herds typically have achieved a degree of control of contagious pathogens (those infections of long duration and prolonged elevated SCC) but may have a problem with environmental mastitis. Because environmental pathogens typically have a shorter duration of infection, prevalence of infected quarters at any one time may be low; therefore, random culturing of cows as suggested in high BTSCC herds may be misleading.

When culturing low BTSCC herds, we currently suggest that two groups be sampled:

(a) Individual high SCC/CMT cows. Even in herds with an acceptable BTSCC, it is important to identify cows that may be harboring contagious pathogens in their udders. In those herds experiencing increased cases of clinical mastitis, but where few cows with elevated (>500,000) SCc are found, we suggest selecting individual quarters for culture, based upon a CMT from those cows withthe highest SCC.
(b) Clinical cases. In addition to culturing high SCC or CMT individual cows or quarters, we suggest that cultures be aseptically collected from affected quarters prior to the treatment of clinical cases. In herds where an increase of clinical cases of mastitis has occurred, the samples from affected cows not requiring professional treatment may be frozen and delivered to the practitioner at the time of a herd visit.

Laboratory procedure— Once in the laboratory, 0.01 ml of milk is streaked for isolation on each of 3 media: 5% sheep blood agar[a]; thallium, crystal violet - ferric citrate agar (TK/FC)[b]: and MacConkey's agar[a], and incubated for 24 hours at 35-37C. Blood agar supports growth of the predominant aerobic bacterial mastitis pathogens. TK/FC is selective for *Streptococci* sp, although *Staphylococcus* sp and some Gram negative organisms can grow in limited numbers on this medium. MacConkey's, of course, is selective for Gram negative bacteria, although some yeasts may also grow on it.

In our laboratory, 100 mm diameter petri plates are quartered so that 4 (composite or quarter) samples can be cultured per plate. In situations where sampling technique may be closely standardized and shipment is not a factor, limited experience in our lab suggests that plating 6 samples per 100 mm plate may be feasible.

After incubation, growth characteristics on the 3 media are observed and a presumptive diagnosis of major

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[a] *Difco Labs. Inc., Detroit, MI.*

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pathogens, (i.e., Streptococcus sp, Staphylococcus sp, coliform) is made (Table 1). In addition, Gram staining is very important, especially when a veterinarian is just beginning to culture, to differentiate between Gram positive rods, and cocci, and some Gram negatives and yeasts which may have a similar morphological appearance on blood agar. Culture plates should be examined again at 48 hours for yeasts, *Corynebacterium* sp, and other slow growing organisms.

TABLE 1. Differentiation of common major mastitis pathogens using three media.*

	Blood Agar	TK/FC	MacConkey's
Streptococcus	+	+	–
Staphylococcus	+	–	–
Coliforms	+	±	+

* Gram negative contaminants will grow on all 3 media.

Gram negative rods - For health management purposes, observation of growth on MacConkey's agar implies that either coliforms (lactose fermenters) or other gram negative organisms are present. If further differentiation is required, an outside laboratory can be consulted.

Catalase test - Gram positive cocci (Streptococci vs Staphylococcus) can be readily differentiated by the catalase test. A colony of growth from a blood agar plate is placed on a microscope slide to which 1 drop of 3% hydrogen peroxide is added. *Staphylococcus* sp. contain an enzyme that catalyzes the reaction $H_2O_2 \rightarrow 2H_2O + O_2\uparrow$ where O_2 bubbles are released; whereas Streptococcus does not catalyze this reaction.

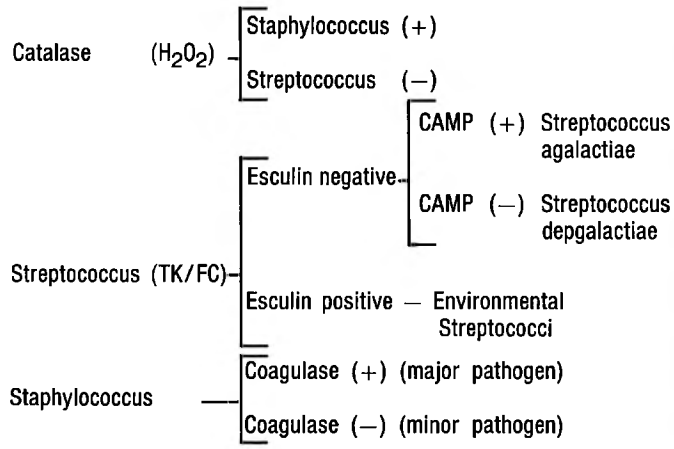
Caution: Prior to testing an organism for catalase it must be determined that it is a gram positive cocci, i.e., other organisms can also cause $O_2\uparrow$ to be liberated from H_2O_2.

Staphylococcus differentiation— It is very important to major contagious pathogens and those which are minor major pathogenic *Staphylococcus* sp (coagulase (+) Staphylococci) cause clot (any degree of clotting is significant) formation when inoculated into rabbit coagulase plasma[a] and incubated 18-24 hours at 37C. Hemolytic patterns on blood agar have been unreliable (in our lab) in determining whether a *Staphylococcus* isolate was going to be coagulase positive or negative.

Streptococcus differentiation— From a diagnostic and control standpoint, it is extremely important to differentiate between *Streptococcus agalactiae* and other Streptococcus sp. *Streptococcus agalactiae* can be eradicated from a herd, while other species cannot be and do not respond to the same control methods. This differentiation is accomplished in 2 steps (Table 2). TK/FC media allows separation of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* from those Streptococci sp that can hydrolyze the carbohydrate,

[a] *Difco Labs. Inc., Detroit, MI.*

TABLE 2. Further differentiation of major mastitis pathogens.



esculin. A black precipitate forms in the media as the esculin positive environmental Streptococci grow. Again, from a practical standpoint, the diagnosis of esculin positive Streptococci provides the basic information needed by the practitioner for institution of control measures without having to further speciate the organism.

The CAMP test is used to determine whether esculin negative Streptococci from TK/FC are *Streptococcus agalactiae* (CAMP+) or *Streptococcus dysgalactiae* (CAMP -). CAUTION: Occasionally *Streptococcus uberis* will be CAMP +; therefore, care must be taken to ensure that a Streptococcus isolate is esculin negative before rendering a diagnosis of *Streptococcus agalactiae* on the basis of a positive CAMP test.

Summary

It can be extremely important for a practitioner involved in a herd mastitis control effort to determine whether contagious and/or environmental pathogens are involved. Monitoring SCC provides indications; however, confirmation can only be made by bacterial culturing of milk. Use of 3 simple media, 5% Sheep blood agar, TK/FC agar and MacConkey's agar, allow a practitioner to determine whether Streptococcus sp, *Staphylococcus* sp, or coliforms are present. The addition of the catalase, coagulase, and CAMP tests further permits the classification of major pathogens so that the herd mastitis control program can be modified for best results.

References

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