Reading On-Farm Bacteriological Culture Results

Mastitis, or inflammation of the mammary gland, is usually caused by bacteria (or another pathogen) invading the udder through the teat end. The severity of mastitis depends on the pathogen involved and the cow's immune response. Producers must be able to identify the causative pathogen in order to make educated treatment decisions. Some treatment options include intramammary antibiotic treatment, close monitoring without antibiotic treatment, early dry off, and systemic support.

Mastitis is the most costly infectious disease and the most frequent reason for antibiotic use on commercial dairy farms. Therefore, management tools that reduce mastitis costs and promote prudent use of antibiotics on dairy farms could be beneficial. The Minnesota Easy® Culture System (University of Minnesota, Saint Paul) is an on-farm bacteriological culturing system that can help veterinarians and producers make strategic treatment decisions based on the pathogen involved in clinical mastitis cases. Cases that yield no bacterial growth when cultured and cases where Gram-negative bacteria are isolated may not benefit from intramammary antibiotics. On the other hand, intramammary antibiotics are often recommended for mastitis caused by Gram-positive organisms. Some examples of Gram-negative pathogens include *Escherichia coli*, Enterobacter, and Klebsiella species. Some examples of Gram-positive pathogens include *Staphyloccus aureus*, coagulase-negative staphylococci, and environmental streptococci (including *Strep uberis* and *Strep dysgalactia*).

A major benefit of on-farm milk bacteriological culture systems is that results can be obtained within 24 hours compared to a week or more for samples sent off to a lab. Withholding intramammary antibiotics for 24 hours after sampling and selectively treating based on the results of the culture has shown no differences in long-term treatment outcomes, including recurrence of clinical mastitis in the same quarter, somatic cell count, milk production, and cow survival for the rest of the lactation after clinical mastitis. Selectively treating cows with antibiotics based on on-farm culture results can decrease antibiotic use by half and tends to decrease milk withholding time without negatively affecting cure rates.

This publication discusses how to read results of on-farm bacteriological cultures grown on the Minnesota Easy® Culture System. Please refer to MSU Extension Publication 3124 *Collecting Milk Samples for Microbiological Analysis* and Publication 3132 *Using an On-Farm Bacteriological Culture System* for tips on collecting aseptic samples and streaking samples for bacteria identification.

Identifying Pathogens Using an On-Farm Culture System

1) **Become familiar with the plate options (Figure 1).** Bi-plates provide two agar (growth medium) types and give general results: Gram-positive or Gram-negative. Tri-plates offer more specific results by showing differences between streps and staphs, which are both Gram-positive pathogen groups. The Minnesota Easy® Culture System tri-plate system will be used throughout this publication.

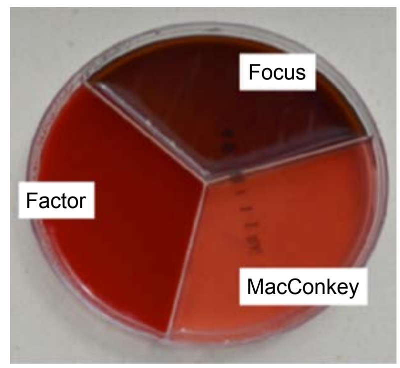


Figure 1. Different agars included in a tri-plate.

2) **Understand the three types of agar media on the tri-plate (Figure 2).** Different types of bacteria will grow on different types of agar.

   a) Red = Factor agar (staph growth)
   b) Dark red = Focus agar (strep growth)
   c) Light pink = MacConkey agar (Gram-negative growth)

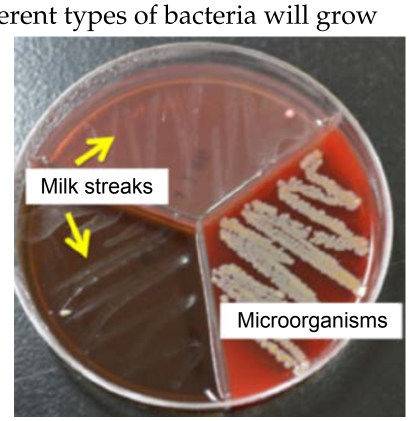


Figure 2. Difference in how milk and microorganisms look after streaking.

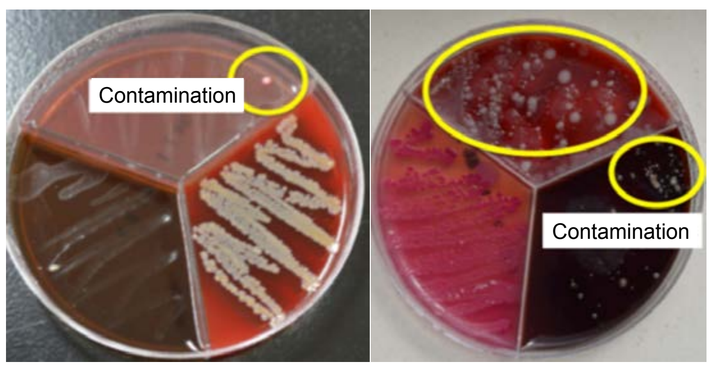
3) **Determine if a pathogen has grown.** A colony, or group of bacteria, is typically yellow, white, or gray and slightly raised above the agar surface. If a mastitis-causing pathogen was present in the milk, the agar typically will have many colonies growing in the pattern that the milk was smeared.

Note: Milk with clots from clinical mastitis or a high fat content is sometimes mistaken as bacterial colonies when streaked on a plate. Keep in mind that milk streaks are typically the same color as the agar and lack the raised colonies, as depicted in Figure 2.

4) **Sometimes plates lack bacterial growth, even from cows with clinical mastitis. If this occurs, consider why.**
   a) **Is the incubator the right temperature?** If the temperature strayed from 99°F, bacteria may have been present but unable to grow because it was too cold or too hot. If this occurred, adjust the temperature and use a new plate to test a new sample from the same or new milk sample from the same cow. Some incubators take time to adjust temperatures, so leaving it on even when samples aren't incubating helps ensure that it is ready as soon as a sample is placed inside.
   b) **Has the cow been treated with antibiotics?** Although a cow may still be showing clinical mastitis symptoms after treatment, milk from cows treated with intramammary antibiotics typically will not show growth until at least a week after the treatment was stopped.
   c) **Was the milk plated correctly?** Milk samples must be gently shaken before plating to distribute the pathogen throughout. Enough milk must be sampled and plated to get adequate growth on the plate. Keeping the no-growth plate in the incubator for another 24 hours may allow growth if a low amount of pathogens were present on the plate.
   d) **Did the cow take care of it herself?** The cow's immune system may have defeated the bacteria before the sample was taken. Even if a cow is still showing clinical signs of mastitis, she may have already experienced a bacterial cure; her body may be still recovering from the trauma of having mastitis and so still shedding some clots and flakes. Also, bacteria may be present in the milk sample but not at a concentration high enough to grow on the plate, meaning the cow is in the process of fighting off the infection and has decreased the number of bacteria present. Sometimes Gram-negative samples result in no growth plates because they typically do not stay in the udder for long periods of time.
   e) **Did you sample a non-clinical cow?** Culturing subclinical mastitis cases, as determined by somatic cell counts, is sometimes recommended to determine what pathogens are affecting the herd so that management changes can be made. However, treating subclinical mastitis cases is not recommended (unless otherwise suggested by a veterinarian) because the cow will often take care of the bacteria without ever becoming clinical. *Staph aureus* is notorious for coming and going in chronically infected cows, so it is possible that the sample simply was not timed correctly to catch it.

Note: No-growth samples are free of contamination, so this indicates good sampling and plating technique.

5) **Determine if there is contamination (Figures 3 and 4).** Contamination typically takes one of three forms: three or more different pathogen types grow on the same plate; a pathogen that does not cause mastitis grows on the plate; or just one or two colonies grow on the plate. Contamination typically comes from the sampler's hands or the parlor environment. Ensuring proper sampling and plating procedures will help prevent contamination.



Figures 3 and 4. Different contamination types and appearances.

6) **Identify the bacteria group based on the agar growth found.**
   a) MacConkey (pink) agar only (**Figure 5**):
      i) Gram-negative bacteria: Treating Gram-negative bacteria with antibiotics could actually cause more harm than good. Antibiotics cause Gram-negative bacteria cell walls to rupture, which can release harmful endotoxins into the cow's bloodstream. These toxins are often what cause mastitic cows to become "toxic" or "systemic"

mastitis cases. Consult your veterinarian for these cases to evaluate the best option for each individual case.

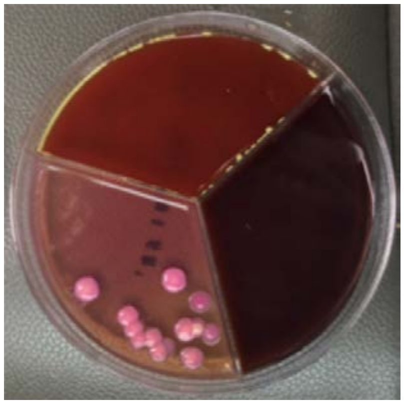


Figure 5. Gram-negative bacteria growth.

b) Factor (red) agar only (**Figures 6 and 7**):

i) Staph species or other non-strep, Gram-positive bacteria.

ii) After determining there is growth in the Factor agar, hold the plate up to light to determine if there is hemolysis, or rupturing of red blood cells, present in the agar. Hemolysis looks like clear rings around the bacterial colonies. *Staphylococcus aureus* produces hemolysis when it breaks down the components of the Factor agar, but coagulate-negative staphs (all other mastitis-causing staph species besides *Staph aureus*) do not. However, the absence of hemolysis does not completely eliminate the possibility of *Staph aureus*. Sometimes, hemolysis does not appear in the first 24 hours of incubation.

Figure 6. Staph species or other non-strep, Gram-positive bacteria growth.

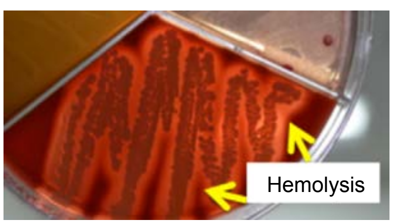


Figure 7. *Staphylococcus aureus* displaying hemolysis on Factor agar.

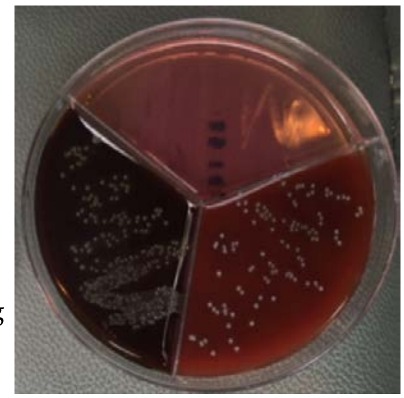
c) Focus (dark) agar only:

i) Strep species. These include *Strep. agalactiae, S. dysgalactiae, S. uberis*, and environmental strep, and Enterococcus species, to name a few. (Photo source: University of Minnesota Diagnostic Laboratory, http://dairyknow.umn.edu/topics/milk-quality/minnesota-easy-culture-system-user-s-guide/)

d) Both Focus (dark red) and Factor (red) agar:

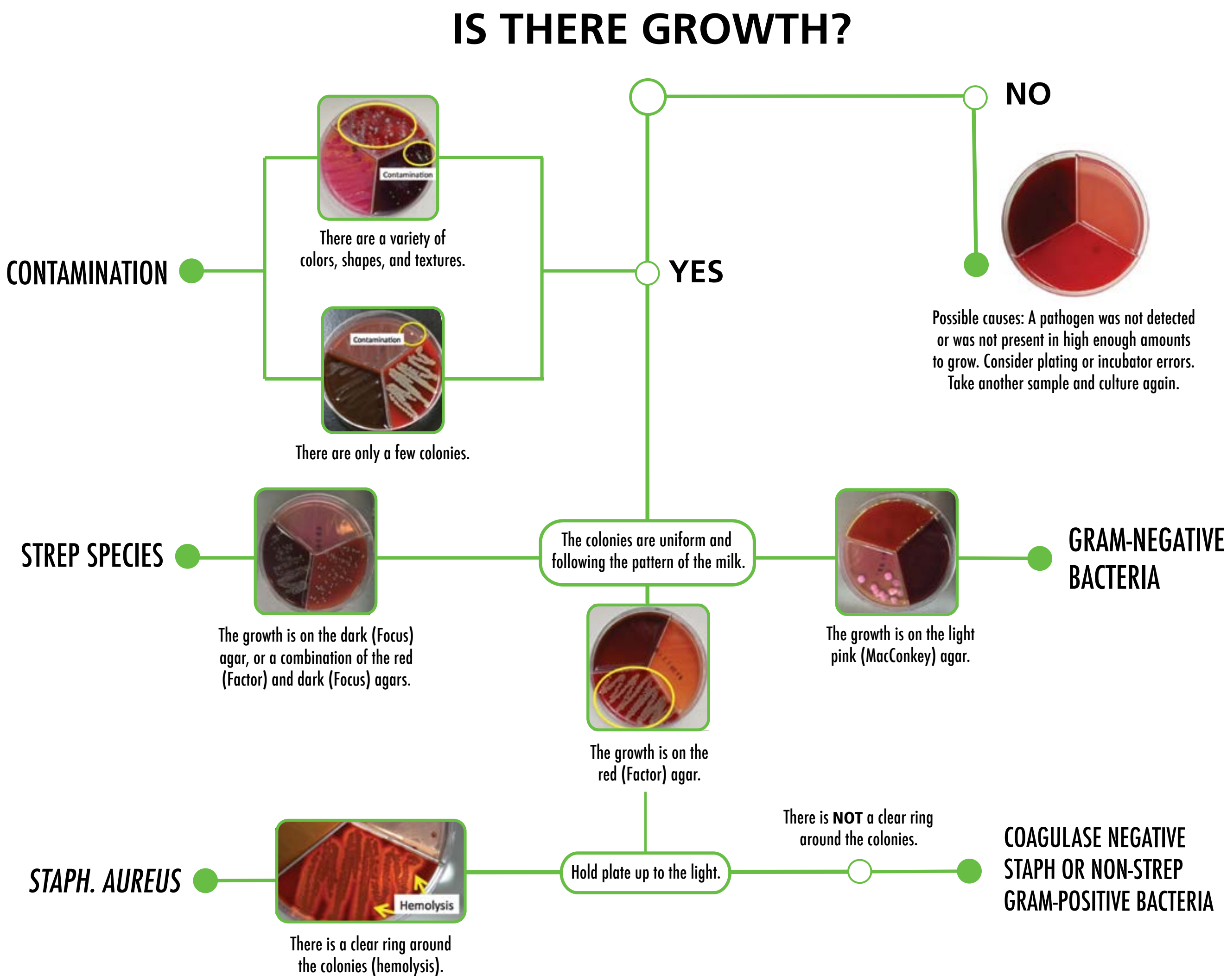
i) Strep species

ii) *Strep agalactiae*: Although *Strep agalactiae* is rarely observed on farms now, it used to be a common, contagious, mastitis-causing pathogen and still could appear on farms. If there is growth in both the Focus agar and Factor agar, hold the sample up to the light. If there are lighter rings (hemolysis) around the colonies on the Focus agar, this indicates *Strep. agalactiae.* If this pathogen is detected, consult your veterinarian because this is a pathogen that typically responds well to treatment.



Applying the Results

- Always consult with a veterinarian for specific treatment recommendations.
- *Staphylococcus aureus* and *Streptococcus agalactiae* are contagious pathogens.
    - Contagious pathogens are spread from cow to cow. In addition to a veterinarian's treatment recommendations, consider evaluating parlor prep procedures, using dry cow therapy, and implementing fly-control techniques.
- Gram-negative bacteria are typically environmental pathogens.
    - Environmental pathogens spread through the cow's interaction with the farm facilities and include coliforms. Coliforms signify that the pathogens come from the cow's manure. In addition to a veterinarian's treatment recommendations, consider evaluating bedding cleanliness, alley cleanliness, and pasture mud levels; fencing off ponds and creeks; and maintaining clean and dry areas for cows to lie down.



Conclusions

On-farm bacteriological culturing can provide many benefits to the dairy farmer, including reduced antibiotic use, the ability to improve treatment success by treating the right bug with the right drug, and the chance to better understand what pathogens are present within the herd in order to make management changes. On-farm bacteriological culturing saves time and money compared to sending milk samples to a laboratory for testing. Producers can get results within 24 hours and develop a specific treatment plan for the infected cow based on veterinary recommendations.

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References

Lago, A., S. M. Godden, R. Bey, P. L. Ruegg, and K. Leslie. 2011a. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. J. Dairy Sci. 94:4441–4456.

Lago, A., S. M. Godden , R. Bey , P. L. Ruegg , and K. Leslie. 2011b. The selective treatment of clinical mastitis based on on-farm culture results: II. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. J. Dairy Sci. 94: 4457-4467.

Pol, M., and P. L. Ruegg. 2007. Relationship between antimicrobial drug usage and antimicrobial susceptibility of Gram-positive mastitis pathogens. J. Dairy Sci. 90:262–273.

Pyörälä, S., L. Kaartinen, H. Käck, and V. Rainio. 1994. Efficacy of two therapy regimens for treatment of experimentally induced *Escherichia coli* mastitis in cows. J. Dairy Sci. 77:453–461.