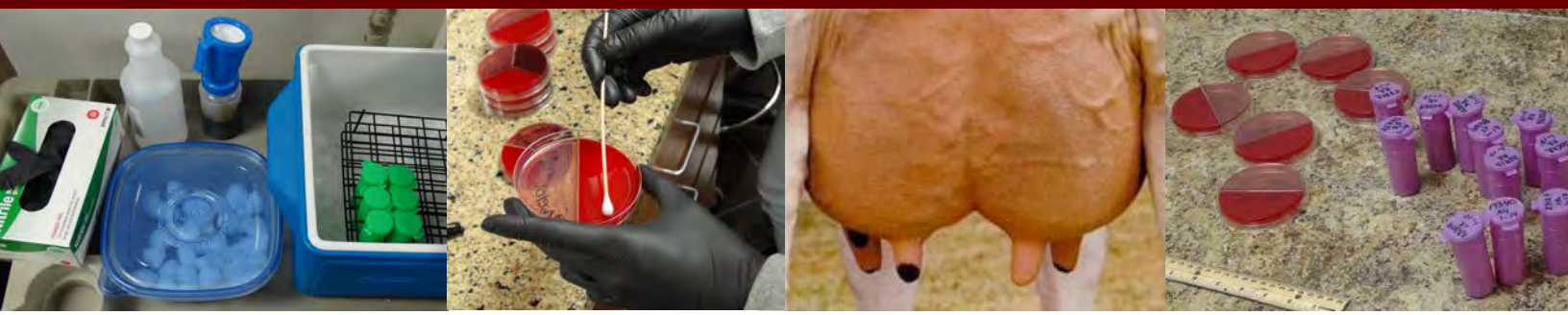

Minnesota Easy™ Culture System User's Guide

UNIVERSITY OF MINNESOTA

VETERINARY DIAGNOSTIC LAB

Minnesota Easy™ Culture System User's Guide

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Introduction

The Minnesota Easy™ Culture System was developed to identify mastitis-causing pathogens in milk samples. The system is based upon commonly used, relatively simple microbiological techniques. The Minnesota Easy™ Culture System is recommended for culturing milk from clinical mastitis cases (visibly abnormal milk).

Brief History of Minnesota Easy™ Culture System

Milk culturing is a laboratory procedure that allows the identification of disease-causing bacteria present in a milk sample. Culturing of milk samples by a diagnostic laboratory is considered to be the "gold standard" for identifying the cause of either clinical or subclinical mastitis. Professionally trained personnel in diagnostic laboratories use a variety of culture and other confirmatory techniques to very accurately identify the type of bacteria present in a sample. Some of these culture techniques are simple and inexpensive and can readily be done on-farm. The purpose of an On-Farm Culture System is to eliminate the need to transport the milk from clinical mastitis cases to a laboratory located some distance away. This allows for positive culture results to be available in as little as 18 hours.

Done properly, On-Farm Culture Systems can provide dairy producers with a quick, simple, and inexpensive way to identify the likely bacterial cause of clinical mastitis. This information can then be used in guiding clinical mastitis treatment decisions.

A variety of On-Farm Culture Systems have been available to dairy producers for a number of years. The Minnesota Easy™ Culture System was developed by faculty at the Laboratory for Udder Health at the University of Minnesota College of Veterinary Medicine. Although there are a number of components to any culturing system, the type and quality of the selective media used is perhaps the most essential component.

The Minnesota Easy™ Culture System utilizes three types of culture media: Factor™, MacConkey and MTKT™.

- **Factor™** media selects for Gram-positive bacteria such as *Staphylococci*, *Streptococci, Bacillus,* and *Corynebacteria.*
- **MacConkey** media selects for Gram-negative organisms. The most common Gram-negative mastitis organisms belong to a group of organisms known as Coliforms. Examples are *E. coli* and *Klebsiella*.
- **MTKT™** media select for *Streptococcus* and *Streptococcus*-like bacteria, such as *Enterococcus* and *Aerococcus* species (Tri-plate only).

While the above medias select for a particular type/s of common mastitis-causing bacteria, other organisms that cause mastitis can also grow on the media. For example, yeast will grow on Factor™ media, *Prototheca* (an algae) will grow on Factor™ or MacConkey media, and *Bacillus* (a Gram-positive bacteria) will occasionally grow on MacConkey media. However, the Minnesota Easy™ Culture System is not designed to identify non-bacterial organisms that cause mastitis, such as yeast and *Prototheca*. Using these selective medias as directed allows users of the Minnesota Easy™ Culture System a high probability of identifying the most common mastitis-causing organisms.

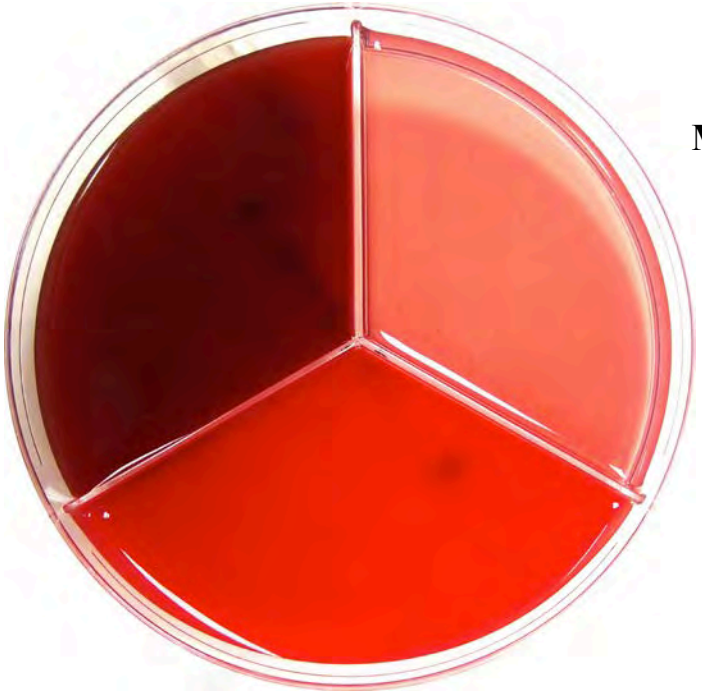
Bi-Plate

Factor™ Media
Gram-positive growth only

MacConkey Media
Gram-negative growth only

Tri-Plate

MTKT™ Media
Strep and *Strep*-like growth only



MacConkey Media
Gram-negative growth only

Factor™ Media
Gram-positive growth only (includes *Staph* & *Strep*)

This manual will provide instruction on how to set up your Minnesota Easy™ Culture System laboratory and review proper laboratory techniques and procedures. In addition, this manual provides introductory training in using the Minnesota Easy™ Culture System to identify the most common bacteria found in milk samples. A commitment to following quality techniques is required to get quality test results. You need to be familiar with and understand the material in this manual to have success using the Minnesota Easy™ Culture System.

Important Resources for Setting Up an On-Farm Culture Laboratory

Your local herd veterinarian is an important resource to help you with your on-farm laboratory. Veterinarians have extensive training in clinical microbiology and many veterinary clinics have excellent laboratory facilities. Your veterinarian can assist you with your equipment setup, provide training in sampling technique, verify results, and most importantly, help you to properly interpret your results. At the University of Minnesota College of Veterinary Medicine, we consider your local veterinarian to be an important component of a successful On-Farm Culture System.

The Laboratory for Udder Health at the University of Minnesota College of Veterinary Medicine is also available to help support you in the proper use of the Minnesota Easy™ Culture System. We encourage you to visit our website for additional resources and up-to-date information.

Laboratory for Udder Health
University of Minnesota College of Veterinary Medicine
Veterinary Diagnostic Laboratory
1333 Gortner Avenue
Saint Paul, MN 55108
Phone: 612-625-7053 (Lab for Udder Health)
800-605-8787 (Veterinary Diagnostic Lab)
Email: mastlab@umn.edu
http://www.vdl.umn.edu/udderhealth/

Additional Resources on Mastitis Prevention and Control

University of Minnesota Dairy Extension - Milk Quality and Mastitis Website
http://www1.extension.umn.edu/dairy/milk-quality-and-mastitis/

University of Wisconsin - Milk Quality Website
http://milkquality.wisc.edu/

National Mastitis Council Website
http://www.nmconline.org/

Laboratory Setup

On-Farm Laboratory Area
Set up your incubator and culture plating space in a dedicated area for your on-farm laboratory. A counter or horizontal work surface that can be easily disinfected and kept free of clutter is needed. This area should ALWAYS BE KEPT CLEAN in order to minimize the risk of accidental contamination of the culture plates, which may result in false positive or contaminated results. Never place food or drink near the laboratory area. Workers should always wear new, clean disposable gloves when working with mastitis samples or culture plates and wash their hands when the work is completed. The on-farm lab area should also be insulated against large fluctuations in room temperature and should be free of drafts.

Incubator Setup and Operation
The purpose of an incubator is to provide a controlled environment that allows bacteria to grow and multiply. The environment in an incubator is controlled for warmth and moisture.

Mastitis bacteria grow best at body temperature, therefore your incubator must maintain its temperature at 37°C (98.6°F). Your incubator should have an easy-to-read thermometer that allows you to monitor that your incubator is operating properly. Mastitis bacteria grow best when the relative humidity is about 75%. This is easily achieved by having a tray filled with water in the bottom of the incubator. Be sure to add water to your incubator often.

Without the proper temperature and humidity, disease-causing bacteria may fail to grow in your incubator, leading to a false negative result of “No Growth”. It is critical you properly set up, monitor and maintain the incubator in your on-farm laboratory. Temperature and water level in the incubator should be checked daily.

It is recommended to set up the incubator approximately one week before use and monitor the temperature at various times of the day to ensure the temperature is constant. One factor that may affect the temperature is the location of the incubator. Ideally the incubator should be set up in an area where the temperature remains fairly constant. If the incubator is located near an outside door or in an unheated location the temperature will fluctuate as the ambient temperature fluctuates. If you are not culturing samples every day DO NOT turn the incubator off. Leave the incubator on, as it will maintain the temperature better, rather than turning the incubator off and on and having to wait until the temperature is constant before being able to incubate a culture.

Incubator Options
A commercially manufactured Laboratory Incubator is available from a variety of laboratory supply sources. There are many options (size and price) to choose from. Please contact your herd veterinarian or the Laboratory for Udder Health at the University of Minnesota College of Veterinary Medicine if you have questions on which incubator is right for your dairy.

As a low-cost entry incubator, many dairies have successfully modified a commercial Egg Incubator. Egg Incubators hold the proper temperature and are of sufficient size to incubate a small number of plates.

Record Keeping
Complete and accurate records are an essential component for any laboratory. We recommend a written log book be kept at the incubator. At a minimum you should record the Sample Date, Cow ID, Affected Quarter, and the final Culture Result. You may wish to capture additional information that will help you with quality control in your on-farm laboratory. An example Culture Log is shown here:

Date	Cow ID	Quarter	Plated by (initials)	Culture Results	Plate Read by (initials)	Incubator Temp	Moisture Check

Culture results may also be entered in your computer records system (such as DairyComp 305) to help you monitor what organisms are causing mastitis on your dairy, and analyze treatment success or failure. Your veterinarian can help you design a consistent scheme for recording culture results.

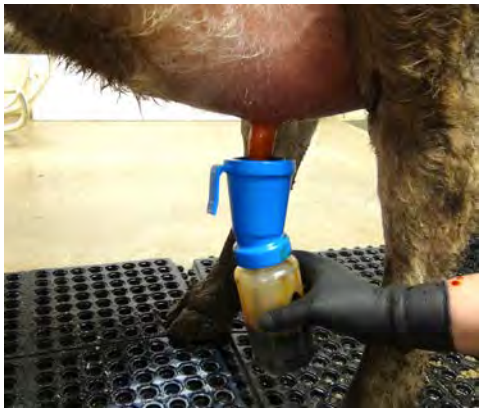
Supplies
- Disposable gloves
- Milk sample vials – 2-ounce plastic flip-top sterile sample vials
- Cotton balls or gauze pads soaked in 70% alcohol
- Cooler with ice or freezer packs
- Racks for holding sample vials
- Disinfectant for cleaning teats (effective germicidal teat dip)
- Paper towels or individual cloth towels
- Waterproof marking pen
- Sterile disposable cotton-tipped swabs
- Minnesota Easy™ Culture System Plates (Bi-plates or Tri-plates)
- Surface disinfectant
- Waste container

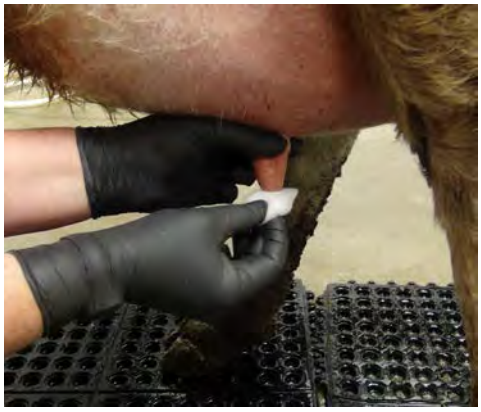
Clean, disposable gloves should be worn anytime you are collecting and plating samples, and reading or handling the culture plates. Milk sample vials are sterile until opened, and should remain closed until the milk sample is collected. Opening the vial only at collection time will help ensure a clean, uncontaminated sample is obtained. Cotton balls or three-inch gauze pads soaked in alcohol can be kept in a clean container with a tight-fitting lid (such as Tupperware) for use when collecting samples. Sterile disposable cotton-tipped swabs should be stored in a clean dry place. Their packaging should not be opened until you are ready to plate a sample. Open the packaging at the wooden stick end so that when you remove a swab from the packaging you do not touch the cotton-tipped end. Unused swabs should be kept in the original packaging and placed inside a ziplock bag or container with a tight-fitting lid. Unused media plates should be stored upside down (lid side down) in the refrigerator. Media should not be used if the expiration date has passed. Do not freeze the media plates. Used plates, swabs, milk samples and vials should be disposed of properly, according to local regulations.

Employee Training
Proper training of all people involved in culturing milk samples is critical to ensuring the success of your On-Farm Culture System. Training should cover equipment maintenance, proper sample collection technique, plating the samples, record keeping, reading the plates and interpreting the results. Periodic retraining is also necessary. We suggest one person be in charge of the lab, monitoring accuracy of results, and training/retraining people to collect and culture samples. If you have any questions about how to organize your lab, your veterinarian should be able to help you. Otherwise contact the Laboratory for Udder Health or attend a seminar on On-Farm Culture Systems.

Sample Collection & Handling

Milk Sample Collection
To obtain a meaningful milk sample it is important to be very clean, as you do not want to contaminate the sample with organisms from outside of the udder.

	1. Wash your hands and then put on new disposable gloves. Label the sample vial using a waterproof marking pen. Clearly record the date, the cow ID and the quarter from which the sample will be taken. RF = right front, LF = left front, RR = right rear, LR = left rear.
	2. Brush off any loose manure, dirt or bedding particles from the udder and teats. Pre-dip with an effective germicidal teat dip, leaving the dip on for 30 seconds. *If the udder and teats are extremely dirty, thoroughly wash and dry the udder and teats before pre-dipping.
	3. Wipe each teat dry with a single-use paper or cloth towel, paying particular attention to the teat end. Be sure there is no teat dip remaining on the teat, as it will kill bacteria in your milk sample.

	4. Discard 3 to 4 streams of milk on the floor to minimize chances of contaminating the sample with bacteria in the teat canal.
	5. Scrub teat ends vigorously for 10-15 seconds using a cotton ball or gauze pad soaked in 70% isopropyl alcohol. Scrub until the ball or pad comes away clean, using as many as necessary. Scrub far teats first, followed by near teats to avoid re-contaminating teats you have already scrubbed. Use a new swab/s for each teat. Teats should not be dripping with alcohol, as this will also kill any bacteria in your milk sample.
	6. Open the sample vial immediately before the sample is taken, not before. Do not touch the inside of the vial or cap or let the teat end touch the vial. Hold the vial at an angle to keep loose dirt or hair from falling into it. Direct streams of milk into the vial without touching the teat end. Sample as quickly as possible, starting with near teats first, followed by far teats. Fill the vial approximately 1/3 full. Attempting to fill the entire sample vial increases the chance of contamination and the full vial may burst when frozen. Immediately close the sample vial so that it is airtight. Collect milk from each quarter into a separate vial (quarter samples).
	7. Immediately place the sample vial on ice or in the refrigerator. Keep samples on ice or in the refrigerator until plated. Freeze samples that will not be plated within 24 hours.

Common Sampling Errors

Contamination of milk samples with environmental bacteria is a common problem and is caused by not being clean enough when taking the sample. Sources of contamination can be the cow's teat if it is not clean enough, the sampler's hands if they are dirty, or the sample vial itself if the vial is opened before milk is put into it or if the vial is left open and contamination falls into it. Some farms will make a batch of alcohol-soaked gauze in a re-usable tub. This can also lead to contamination if the tub is not kept clean or if the alcohol is allowed to evaporate. With proper technique, fewer than 5% of individual quarter samples should be 'contaminated'. If contamination is encountered more frequently, then review sampling technique, sample handling, and culturing technique with farm staff involved. Frozen milk samples may also be submitted to a microbiology laboratory for confirmatory testing.

It is strongly recommended to collect milk from individual clinically affected quarters. Do not collect composite samples (commingled milk from 2 or more quarters) because interpretation of results is difficult: If more than two environmental pathogens are cultured from a composite sample, it is impossible to determine whether the organisms came from multiple infected quarters or if the sample was contaminated (results unreliable).

Contamination of milk samples with disinfectants is sometimes a problem that can lead to no-growth culture results. A common way this happens is when teat dip or alcohol gets into the sample. This happens when the teat is not dried off thoroughly before the sample is taken or teat dip is on your gloves. This problem may be partially avoided by discarding 2 to 3 squirts of milk on the floor immediately prior to collecting the milk sample.

Here are some tips to help ensure you get a CLEAN sample from the cow:

- Sampling milk from a full udder usually allows easier collection of the sample, making it less likely to contaminate the sample.
- Sampling in a clean location reduces the likelihood of contaminants falling into the sample. Because of this the parlor is the ideal place to take milk samples.
- Plating the samples as soon after collection as possible also minimizes the effect of contamination.
- Be aware of manure on your clothing or hands and wash or change if this leads to manure in the samples.

Examples of factors that increase the chances of contaminated samples:

- Re-using culture plates, sample vials, or cotton swabs.
- Extremely dirty udders often result in contaminated samples.
- Collecting samples in a place where manure can splash into the sample.
- Manure or dirt on hands and sleeves of sampler.
- Leaving milk sample vials unchilled for more than 1 hour after collection (before plating).
- Leaving sample vials or culture plates uncovered longer than necessary.

Sample Labeling

Accurate labeling is very important to getting useful information from this system. We recommend you label each milk sample vial and each culture plate with the date, Cow ID, and quarter affected. Use a permanent marker so that the condensation in the incubator or freezer will not smear your identification, making the labels illegible.

Storing Samples
If possible, milk samples should be plated immediately after they are obtained. If you are not able to immediately get the sample to the lab for plating, the length of time it can be stored depends upon the temperature of the sample. The following time guidelines should be used when storing samples.

- At room temperature – Less than 1 hour
- At refrigerator temperature – More than 1 hour, less than 2 days
- At freezer temperature – More than 2 days, less than 60 days

If you are freezing samples for extended periods it is sometimes helpful to group the samples by week in a plastic bag or box so that they can be easily found if needed.

Culturing Procedures

Sample Preparation

If the sample cannot be plated immediately (within 1 hour) refrigerate or freeze the sample until you have time to plate it. This is critical to obtaining quality results. If the sample is frozen, allow it to thaw completely in the refrigerator before plating. To mix the sample, first make sure the lid is properly sealed. Mix each sample vial well by gently inverting the sample approximately 15 times.

Plating Procedure

1. Wash hands and then put on new disposable gloves.
2. Turn the plate upside down and label each sample on the bottom of the plate (label with the cow ID, quarter, and date).
3. Use a new sterile cotton swab and a new plate. Never re-use a plate, even if no previous growth was detected. Never use a wet, dirty, or used cotton swab, as you will not get reliable results.
4. Avoid touching the plate or cotton end of the sterile swab with your fingers or to any surface as this will result in contamination. (Open cotton swab packages such that the cotton end remains covered and only the stick is exposed.)
5. Place a sterile cotton swab end in the milk sample, rolling the non-cotton end of the swab between index finger and thumb, for approximately 8 to 10 seconds, or until the swab becomes completely saturated with milk.
6. Try to avoid plating clumped milk. You can roll the swab on the side of the sample vial to knock milk clumps off the cotton tip. If milk clumps happen to get onto the plate use a marker on the bottom of the plate to note where they are so as not to confuse them with bacterial growth.
7. Swab the sample onto each of the Minnesota Easy™ Culture System media. To do this, the media plate should be lid down on the counter, pick up the media side of the plate in the palm of your non-dominant hand. Take your cotton swab that you saturated with the milk sample and streak the milk over the entire surface of the plate section using one of the patterns shown on the next page.
8. Re-dip the swab in milk between each section of the culture plate.
9. Once the plate has been swabbed, place the lid back on the plate and immediately re-seal the lid on the milk sample. Freeze the milk sample immediately (in case of need for confirmatory testing at a later date).
10. Place the plate in the incubator upside down (media facing down or place the plate on its lid) so that any condensation on the lid will not drip onto the plated sample.
11. The plates should remain in a 37° C incubator for up to 48 hours. After 18 to 24 hours you will read the plate for the first time. Refer to the Interpreting Culture Results section of this manual for information on reading plates.
12. You need to be clean here as well. Be aware of ways manure or dust can get into your culture plates; including from your sleeves, clothing, hands, hair, or surroundings. Disinfect the surface of the workspace and clean up spills immediately.

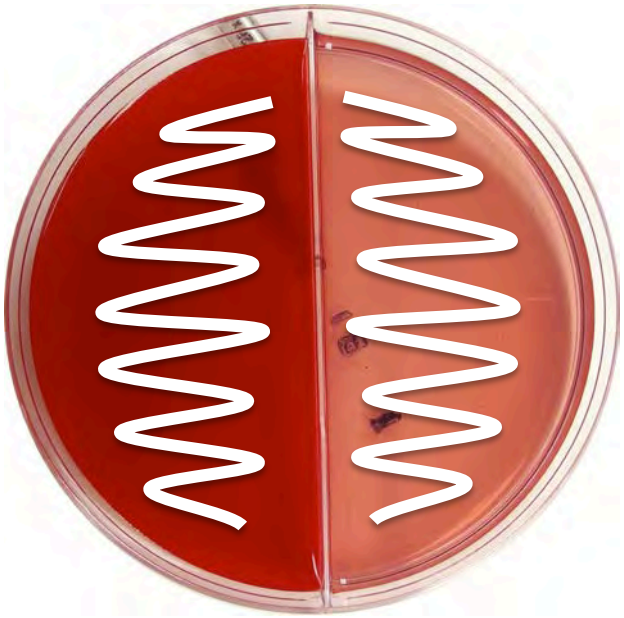
Swabbing pattern

Be sure to re-dip the swab between each section of the Tri- or Bi-plate. This will help ensure enough sample is put on the plate to get accurate results. When using the Tri- or Bi-plates, we recommend swabbing in the following sequence:

Bi-plate:	1. Factor™	2. MacConkey	
Tri-plate:	1. Factor™	2. MacConkey	3. MTKT™

Bi-Plate:

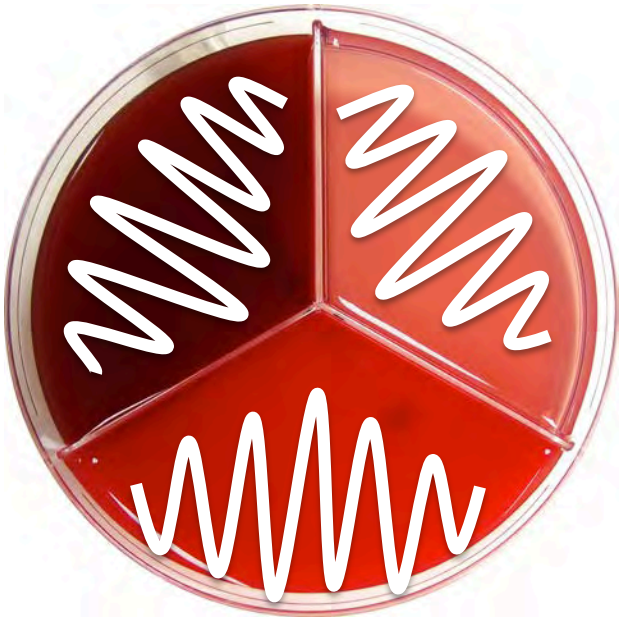
1st swab Factor™ Media



2nd swab MacConkey Media

Tri-Plate:

3rd swab MTKT™ Media



2nd swab MacConkey Media

1st swab Factor™ Media

Figure 1. Swabbing Pattern and Order on Bi-plate and Tri-plate.

Incubator considerations

- Be sure plates are clearly labeled
- Place the plate in the incubator upside down (media face down or place the plate on its lid).
- The incubator should be set to 37°C (or 98.6°F).
- Samples need to incubate for at least 18 hours, but no longer than 48 hours.
- Be sure a full open container of water or wet towel is in the incubator. Humidity is important to providing an environment conducive to bacterial growth.

Saving Samples

After plating, you may wish to save samples for re-culturing or sending in to a laboratory for confirmation. Milk samples can be stored in a refrigerator for 1 to 2 days. It is a good habit to freeze milk samples immediately after plating to save them. We recommend labeling the sample vial, and grouping frozen samples by the week collected to aid in finding the sample when needed. In general, milk samples can be stored frozen for up to 8 weeks in a properly operating freezer.

Interpreting Culture Results

The purpose of the Minnesota Easy™ Culture System is to identify the type of bacteria present in milk from individual quarters from cows with clinical mastitis. Cows with clinical mastitis will have visibly abnormal milk. It is not recommended to use the Minnesota Easy™ Culture System to screen for contagious mastitis in cows with normal milk. Culturing of composite samples (comingled milk from more than one quarter) is also not recommended, as interpretation of results becomes difficult, particularly for environmental pathogens. The Minnesota Easy™ Culture System can help users identify the type of bacteria present in a quarter milk sample based upon the use of selective media, which allow certain types of bacteria to grow, while also preventing the growth of other types of bacteria. Depending on which media bacteria are growing on, users can draw conclusions about the type of bacteria present in the sample.

It takes time for bacteria to grow on the media plates. Some bacteria grow much more quickly than others. You can first check your plates at 18 hours and again at 24 hours to look for bacterial growth. Typically, you will see bacterial growth at the 24-hour time. Regardless of whether or not you see any bacterial growth at 24 hours, it is recommended to incubate all plates for 48 hours. Certain organisms may not show up until 48 hours, including *Trueperella pyogenes* (formerly known as *Arcanobacterium pyogenes*), *Prototheca,* and yeast. Nearly all mastitis-causing bacteria will show growth in the Minnesota Easy™ Culture System by 48 hours. After 48 hours, the chances of contaminant bacterial growth increases and the media begins to dry out, so you should always make a final reading by the 48-hour mark. After reading your plates and recording the results, be sure to dispose of used plates according to local regulations.

Interpreting Bi-Plates

Growth vs. No Growth
This is the simplest level of diagnosis and can be determined with either a Bi-plate or a Tri-plate. In general, growth in any section of the plate indicates bacteria are present in the milk and antibiotic therapy may be indicated. This may not be true if three or more types of bacteria are present (see Contaminated Results). A No Growth result may indicate that the bacterial infection is no longer present and antibiotic therapy is not likely to be beneficial.

Gram-positive vs. Gram-negative Growth

It is also useful to know if the type of bacteria present is Gram-positive or Gram-negative. Gram staining is a simple technique that scientists may use in the laboratory. Certain types of bacteria will retain Gram stain (making them Gram-positive) while other types of bacteria do not retain Gram stain (making them Gram-negative). This level of diagnosis is useful because some mastitis infections caused by Gram-negative bacteria may not need intramammary antibiotic therapy. Before implementing an On-Farm Culture System you should discuss with your veterinarian how to use the results to make treatment decisions.

With the Minnesota Easy™ Culture System, the Factor™ media (bright red) is designed to only grow Gram-positive bacteria and MacConkey media (clear/pink) is designed to only grow Gram-negative bacteria.

Figure 2. Growth on the Factor™ media indicates Gram-positive bacteria.

Figure 3. Growth on the MacConkey media indicates Gram-negative bacteria.

Staphylococcus aureus

Minnesota Easy™ Culture System users may also be able to identify bacterial growth caused by *Staphylococcus aureus. Staph aureus* colonies will appear on Factor™ media as creamy, greyish-white or golden yellow, with a clear area of hemolysis around the colony. The zone of hemolysis may be small at 24 hours, but is often much larger by 48 hours. If there is Gram-positive growth but no zone of hemolysis on the Bi-plate, it is likely the organism is something other than *Staph aureus* (e.g. other *Staph* or *Strep* species). However, some strains of *Staph aureus* may not show distinct hemolysis and some non-aureus *Staph* species can be hemolytic. Consider confirmatory testing of suspect *Staph aureus* colonies with the tube coagulase test, which can be performed on-farm or in a diagnostic laboratory. Consult your veterinarian for advice on treating, segregating (e.g. milking last), or culling cows with *Staph aureus* mastitis.

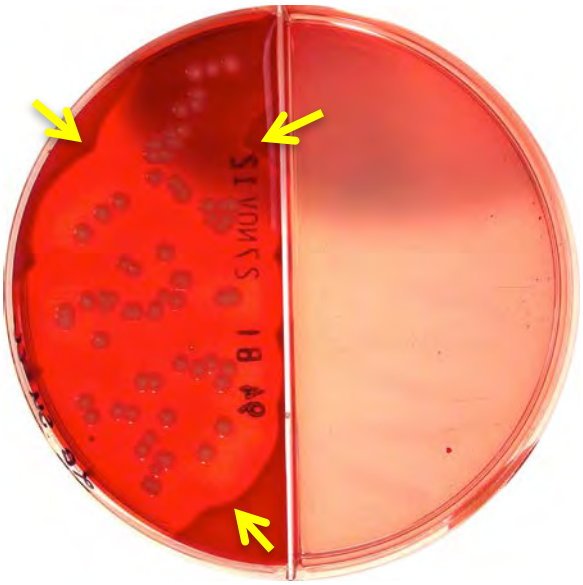


Figure 4. *Staph aureus* on a Bi-plate showing a clear zone of hemolysis (indicated by arrows).

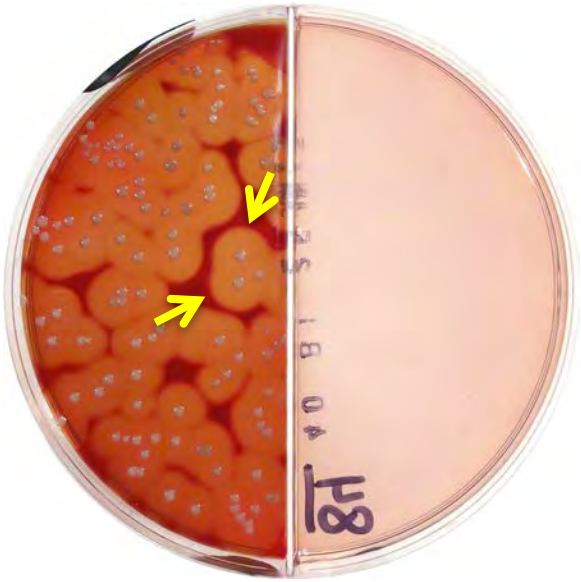
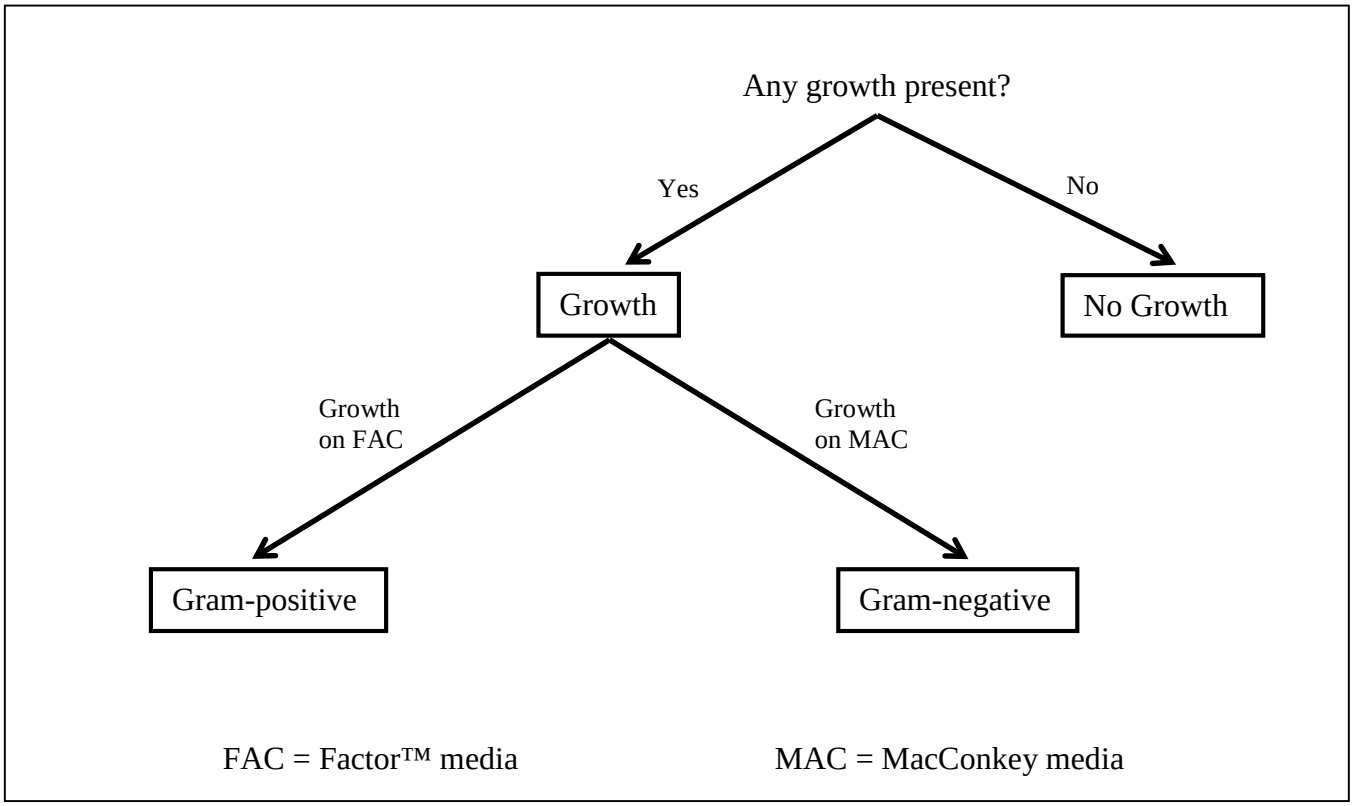


Figure 5. Another example of *Staph aureus*. Zone of hemolysis indicated by arrows.

Flow Chart for Identification of Mastitis Organisms Using the Bi-Plate



Interpreting Tri-Plates

Growth vs. No Growth
This is the simplest level of diagnosis and can be determined with either a Bi-plate or a Tri-plate. In general, growth in any section of the plate indicates bacteria are present in the milk and antibiotic therapy may be indicated. This may not be true if three or more types of bacteria are present (see Contaminated Results). A No Growth result may indicate that the bacterial infection is no longer present and antibiotic therapy is not likely to be beneficial.

Gram-positive vs. Gram-negative Growth
It is also useful to know if the type of bacteria present is Gram-positive or Gram-negative. Gram staining is a simple technique that scientists may use in the laboratory. Certain types of bacteria will retain Gram stain (making them Gram-positive) while other types of bacteria do not retain Gram stain (making them Gram-negative). This level of diagnosis is useful because some mastitis infections caused by Gram-negative bacteria may not need intramammary antibiotic therapy. Before implementing an On-Farm Culture System you should discuss with your veterinarian how to use the results to make treatment decisions.

With the Minnesota Easy™ Culture System, the Factor™ media (bright red) and MTKT™ media (dark red) are designed to only grow Gram-positive bacteria, and the MacConkey media (clear/pink) is designed to only grow Gram-negative bacteria.

Figure 6. Growth on the Factor™ media indicates Gram-positive bacteria.

Figure 7. Growth on both Factor™ and MTKT™ media also indicates Gram-positive bacteria.

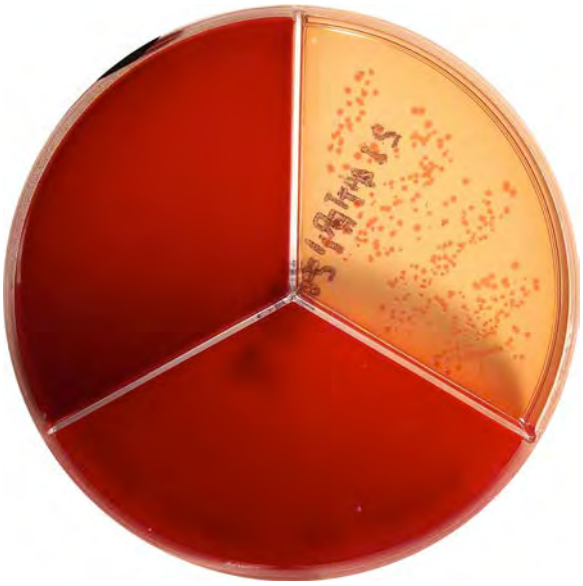


Figure 8. Growth on the MacConkey media indicates Gram-negative bacteria.

Staphylococcus aureus

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Figure 9. *Staph aureus* on a Tri-plate showing a clear zone of hemolysis (indicated by arrows).

Figure 10. Another example of *Staph aureus*. Zone of hemolysis indicated by arrows.

***Staphylococcus* species vs. *Streptococcus* species**

With the Tri-plate, users can also determine whether Gram-positive bacterial growth is caused by *Staph* species or *Strep* species. The MTKT™ media selects for *Strep* growth (only *Strep* and *Strep*-like species will grow). Therefore, if growth is present on both the Factor™ and MTKT™ media, the growth is most likely a *Strep* species. If growth only occurs on the Factor™ media, you can rule out *Strep* as a possible cause (*Staph* is most likely).

Figure 11. Growth on both Factor™ and MTKT™ media indicates likely *Strep* species.

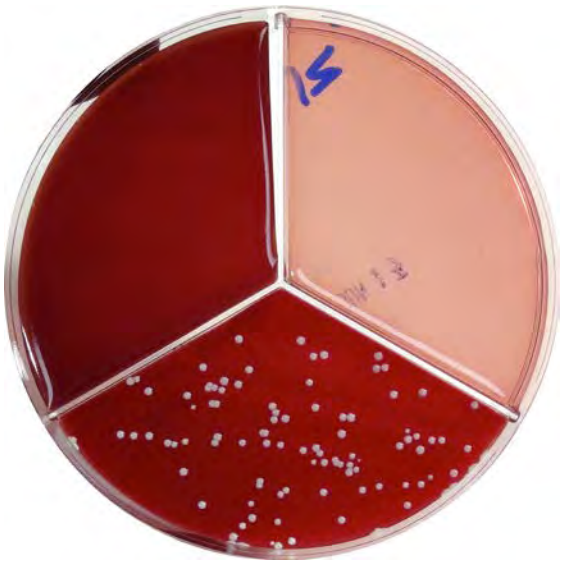


Figure 12. Growth on Factor™ media only indicates likely *Staph* species.

Special Note on *Streptococcus agalactiae*

Strep agalactiae is a contagious mastitis-causing organism that has largely been eradicated from U.S. dairy herds. Although it is unlikely that users would encounter *Strep ag*, the MTKT™ media can be used to identify *Strep ag* based on a zone of hemolysis around the colonies, as shown in the following figure. Since *Strep ag* is uncommon, the ability of users to accurately identify it was unable to be determined in the validation study (see reference on page 19). If you suspect that you have identified *Strep ag*, contact your veterinarian immediately for confirmation and advice.

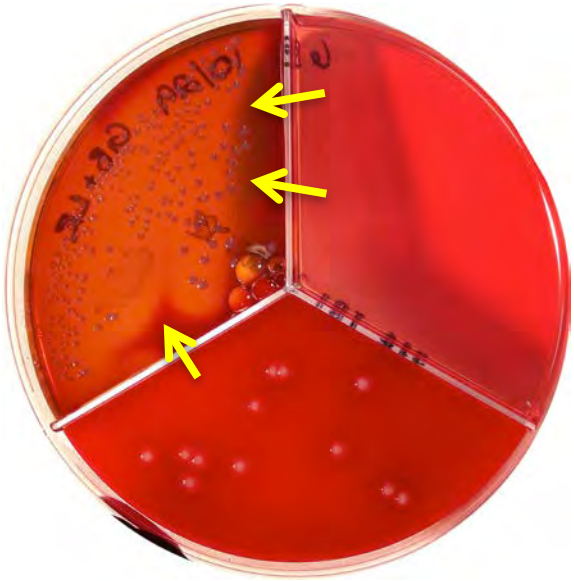
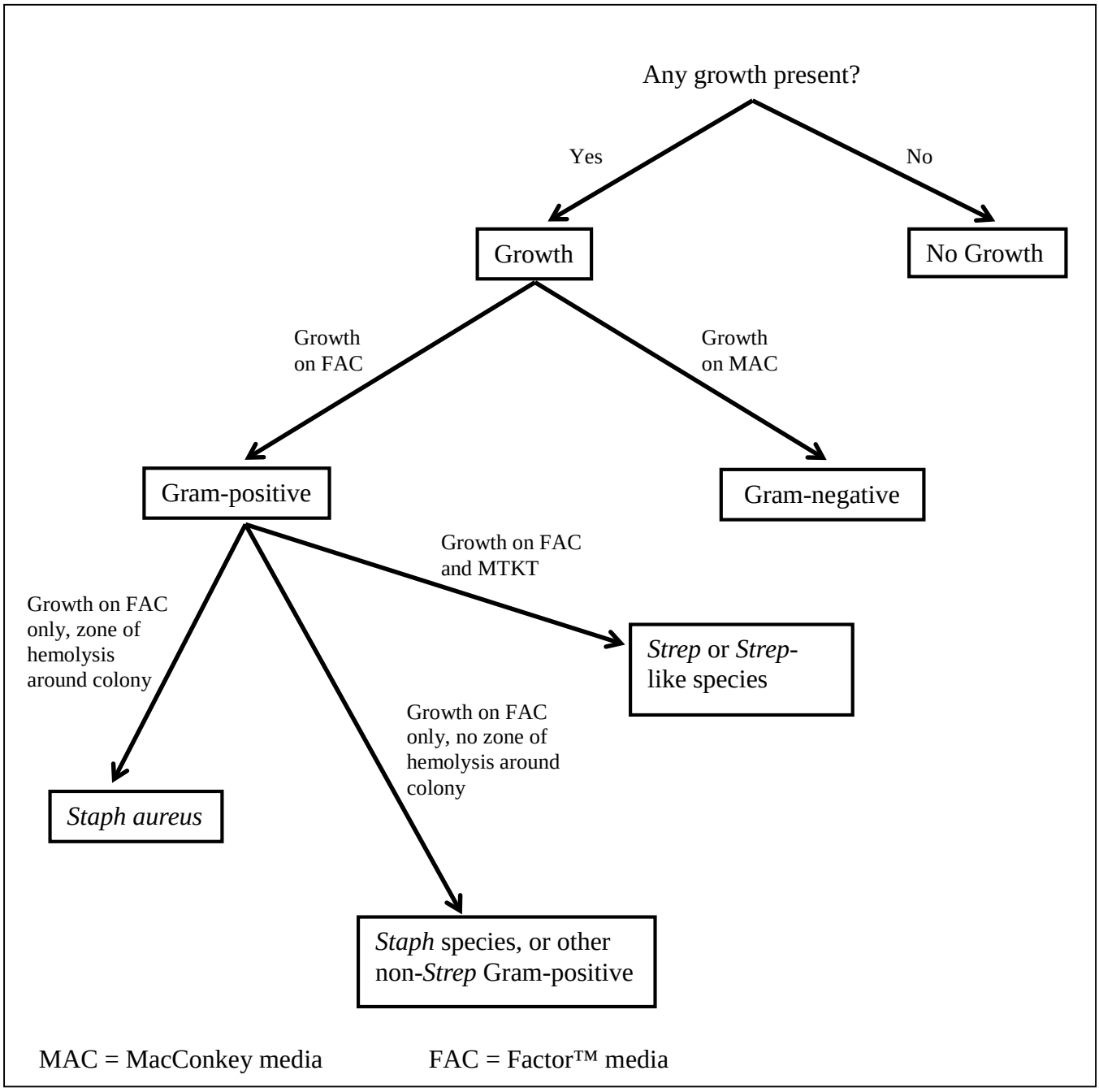


Figure 13. Growth on both Factor™ and MTKT™ media with zone of hemolysis indicates likely *Strep agalactiae*.

Figure 14. *Strep agalactiae* – note that backlighting improves visualization of the zone of hemolysis.

Flow Chart for Identification of Mastitis Organisms Using the Tri-Plate



Further Interpretation

Users may be tempted to try and identify bacterial species beyond the levels of diagnosis discussed in this manual based on what the colonies look like or how they react with the media. For example, if bacteria grows on MTKT™ media, is it *Strep uberis* or *Strep dysgalactiae* or *Aerococcus*, etc.? A validation study was conducted to determine the ability of farm personnel to accurately diagnose the cause of mastitis using the Minnesota Easy™ Culture System. The following levels of diagnosis were evaluated:

- Growth or No Growth
- Gram-positive or Gram-negative Growth
- Genus classification: *Staph* species or *Strep* species
- *Staph aureus* present or not
- Species classification of *Streps* (*Strep agalactiae, Strep uberis, Strep dysgalactiae*, etc.)
- Species classification of Gram-negatives (*E. coli, Klebsiella*, etc.)

In this study, two readers without specialized training (similar to an on-farm reader) interpreted 172 samples at each of the above levels of diagnosis. A trained microbiologist in the Laboratory for Udder Health also interpreted each of the samples and the results were compared for agreement and accuracy. It was found that the most accurate and useful results can be obtained on-farm when using the Minnesota Easy™ Culture System at the following levels of diagnosis:

The Bi-plate system can reliably be used to diagnose:

- Growth or No Growth
- Gram-positive or Gram-negative Growth
- *Staph aureus* present or not

The Tri-plate system can reliably be used to diagnose:

- Growth or No Growth
- Gram-positive or Gram-negative Growth
- Genus classification: *Staph* species or *Strep* species
- *Staph aureus* present or not

However, the Bi-plate and Tri-plate systems were less reliable in reaching more detailed levels of diagnosis for bacterial species other than *Staph aureus* (e.g. other Staph species, *Strep uberis, Strep dysgalactiae, E. coli, Klebsiella*). It is therefore not recommended to use the Minnesota Easy™ Culture System to try to diagnose bacteria at the species level, with the exception of *Staph aureus*. If desired, refer to the Laboratory for Udder Health or your veterinarian's in-house lab for further diagnostics.

For more information about this validation study, please contact the University of Minnesota Laboratory for Udder Health or see: Royster, E., Godden, S., Goulart, D., Dahlke, A., Rapnicki, P., and Timmerman, J. 2014. Evaluation of the Minnesota Easy Culture System II Bi-Plate and Tri-Plate for identification of common mastitis pathogens in milk. J. Dairy Sci. 97:3648-3659.

Contaminated Results

A milk sample is considered "contaminated" when three or more types of bacteria are identified on the Minnesota Easy™ Culture System media. Typically when individual quarter milk samples are collected properly, only a single mastitis pathogen is identified. When a milk sample is "contaminated" it is uncertain which if any of the bacteria found are causing disease and which are merely environmental bacteria that contaminated the milk sample during the collection process. Consideration must be given to the types of bacteria isolated, the number of colonies appearing on the plate, and the stage of infection. Dairy producers are encouraged to consult with their herd veterinarian, and should consider resampling the affected quarters using very clean sampling, handling and plating techniques.

Figure 15. Three or more types of bacterial growth indicate the sample was likely contaminated.

With proper techniques, fewer than 5% of individual quarter samples should be 'contaminated'. If contamination is encountered more frequently, review milk sampling technique, sample handling, and culturing procedures with farm staff involved. Frozen milk samples may also be submitted to a microbiology laboratory for confirmatory testing.

No Growth Results

If no bacterial growth is evident after 48 hours, then the sample is considered a "No Growth." Various sources have reported between 25 – 40% of samples from quarters with clinically abnormal milk will be No Growths. There are four possible explanations for a No Growth result:

1. The quarter sampled was not infected (true negative).
2. The quarter sampled was infected, but the cow's immune system has already responded to the infection and eliminated the bacteria before the sample was taken (true negative). This is common in Gram-negative infections.
3. Equipment failure, or errors in sample collection, storage or culture technique resulted in a false negative result.
4. The type of bacteria or other organism causing the mastitis does not grow under the conditions of the Minnesota Easy™ Culture System (false negative), for example *Mycoplasma* species.

If more than 40% of your on-farm culture results are No Growths, consult with your veterinarian or the Laboratory for Udder Health to determine the cause.

Special Note on *Mycoplasma*

Mycoplasma is a serious contagious pathogen that can cause bovine mastitis. *Mycoplasma* is a unique type of bacteria that only grow under special incubation conditions (e.g. reduced oxygen, specialized media, 5-7 day incubation period), so the Minnesota Easy™ Culture System will not detect *Mycoplasma*. A *Mycoplasma* infection would show up as a "No Growth" with the Minnesota Easy™ Culture System technique. If a quarter persists in showing signs of clinical mastitis (abnormal milk) despite repeated 'No Growth' results using the Minnesota Easy™ Culture System, producers are encouraged to submit frozen aseptically collected samples from the affected quarter/s to a microbiology laboratory for confirmatory testing, including ruling out infection with *Mycoplasma* spp.

Special Note on *Prototheca*

Prototheca organisms can cause clinical mastitis. However, *Prototheca* are not bacteria, rather they are micro-algae. *Prototheca* may grow on Factor™ and/or MacConkey media. Note that the Minnesota Easy™ Culture System was not designed to identify algae. We recommend consulting with your herd veterinarian or the Laboratory for Udder Health for assistance if you suspect *Prototheca* in your herd. If a quarter persists in showing signs of clinical mastitis (abnormal milk) despite intramammary antimicrobial therapy, as well as repeated Gram-positive and Gram-negative growth results using the Minnesota Easy™ Culture System, producers are encouraged to submit frozen aseptically collected samples from the affected quarter/s to a microbiology laboratory for confirmatory testing.

Trouble Reading Plates

Anytime you are uncertain about your results, believe you have an unusual isolate, or the quarter is failing to respond (clinical mastitis failing to resolve), the original frozen milk sample or the media plate can be used as a way to transport the unknown organism to your local veterinarian's laboratory or to the Laboratory for Udder Health for positive identification. However, it is preferred to submit the original frozen milk sample (less preferred to submit the media plate).

If you are having trouble culturing bacteria, or are not certain if you are correct in identifying organisms, it is recommended to re-read this manual and pay close attention to details. It is very important to follow all steps of this guide to ensure the culture system is accurate and treatment decisions are being based on accurate results. For example, how the sample is taken from the cow is just as important as how it is plated and equally important is the maintenance of the incubator. This guide is meant to aid in understanding the entire process of culturing. If you have unanswered questions after reading this manual please feel free to contact your herd veterinarian or the University of Minnesota Laboratory for Udder Health.

On-Farm Laboratory Quality Control

It is a good idea to periodically verify the results of your culturing efforts. There are several approaches that can be used:

- Regularly review plates with your veterinarian when he/she visits the dairy (e.g. once per month).
- Send frozen milk samples to a diagnostic laboratory (such as the Laboratory for Udder Health) to be cultured, and then compare results with those obtained on-farm. It is recommended to send three to five samples every four months for verification.

More frequent verification should be utilized when you are first starting to use the On-Farm Culture System.

Minnesota Easy™ Culture System User's Guide