HOW TO SET UP CULTURE PLATES

Before plating, check that all equipment is present and accessible:
  o Culture plates
  o Sterile swabs
  o Milk samples and sample rack
  o Clean disposable gloves
  o Gauze soaked in 70% alcohol

Clean work space with gauze and 70% alcohol to eliminate environmental bacteria that otherwise might contaminate the culture plates. When setting up culture plates be sure to always wash your hands and wear clean disposable gloves.

Thaw any frozen samples to room temperature. This will require setting the samples out on the lab bench for at least an hour prior to plating. Do not thaw frozen samples using hot water or the microwave.

Label the side of the culture plate with cow ID, affected quarter and the date.

Thoroughly mix the milk sample by inverting it several times. Dip a sterile cotton swab into the sample and wait 5 seconds for it to become saturated with milk. Spread the swab over the media surface using a back-and-forth motion from the top down. Redip the swab in the milk sample for each section of the culture plate. Immediately cover the plate and cap the milk sample after plating to avoid contamination.

Freeze milk samples so they are available for confirmatory testing later.

Lay the plates flat on the work surface for 5-10 minutes to allow the milk to soak into the agar.

Invert all plates so the agar side is up so that condensation that collects on the lid will not drip onto the agar and disrupt bacterial growth.

Place the upside-down plates in the incubator and incubate at 37 degrees Celsius (98 degrees Fahrenheit) for 24 hours. The bacteria will need a minimum of 24 hours to grow before reading results.

Throw away any garbage and disinfect the work space.
HOW TO SET UP CULTURE PLATES

Have all materials ready to use:
Milk samples
Culture plates
Sterile swabs
Gloves
Gauze soaked in 70% alcohol
Biohazard bags

Label the side of a new on-farm culture plate with:
Cow ID
Affected quarter
Date

Thoroughly mix the milk sample by inverting it several times.
How to Set Up Culture Plates

Dip a sterile swab in the milk sample and roll it until swab becomes completely saturated with milk.

Inoculate one section of a culture plate using a zigzag streaking pattern.

Re-dip the swab in the milk sample before inoculating each section of the culture plate.
Inoculate the resting sections of the culture plate.

Place the inoculated culture plates in the incubator upside down (lid facing the shelf) and incubate plates for 18 to 24 hours at 37°C.
The purpose of performing on farm culturing is not only to grow bacteria but to ensure that our diagnosis is correct so that we can determine if antibiotic treatment will be helpful.

**FIRST STEP: ASSESSING BACTERIAL GROWTH**

Bacterial growth takes a minimum of 24 hours in incubation before the plate can be observed. After 24 hours, the first question to ask is: **Is there any growth on the plates?**

**No Growth**

There are several reasons this happens. First, the quarter may not be infected or the infection may already have been cleared by the body’s immune system. Also, improper handling or lab errors may cause no growth. Lastly, fastidious organisms that require special media, such as Mycoplasma, may cause infection but will not grow on standard culture plates.

**SECOND STEP: INTERPRETATION OF BACTERIAL GROWTH**

If there is growth on the plate, the second question to ask is: **How many different types of bacterial colonies are there?**

**Contaminated**

If there are more than 2 types of colonies, the plate has been contaminated. When this happens, the quarter may be resampled using proper collection technique and cultured again.

*If there are 1 to 2 colony types, the number of ‘colony forming units’ should be evaluated.* A ‘cfu’ is a small circular, often raised growth of bacteria.

**Non-significant Growth**

Fewer than 3-5 cfu’s per colony type signifies non-significant growth. This means that the bacteria present on the culture plate are too few in number to be the cause of mastitis.

**True Infection**

When there are 3-5 or more cfu’s per colony type, there is a true infection, and identification can be performed. Sometimes there may be a true infection that has a contaminant. As long as the contaminant has non-significant growth, you can still identify the true infection.
Determining the cause of mastitis is important because not all cases of mastitis benefit from antibiotic therapy. For instance, Gram-Positive bacteria, such as Staphylococcus aureus and coagulase negative behave differently in the cow and have different responses to therapy. Being able to identify between species can help us make appropriate treatment decisions for managing mastitis in our herds. One way to do that is through on farm culturing.

**Biplate**
*Gram-Positive bacteria* (growth only on Factor or Blood agar)

**Triplate**
*Staphylococci* (growth only on Factor or Blood agar)

**Quadplate**
*Staphylococci* (growth on Blood Agar and Factor agar)

**Contamination**
*Contaminated plate* (growth on all agars)
Streptococcus species are a common cause of mastitis and frequently associated with high somatic cell counts, and in some cases clinical mastitis. Streptococci are gram positive organisms that also grow in the environment. Learn how to identify Streptococci on a biplate, triplate and quadplate using selective agars in your on-farm culturing lab.

**Biplate**
*Gram-Positive bacteria* (growth only on Factor or Blood agar)

**Triplate**
*Streptococci* (growth on both Factor and MTKT agars)  
*Streptococcus agalactiae* (zone of hemolysis in MTKT)

**Quadplate**
*Streptococci* (growth on Blood Agar, Factor and MTKT agar)

**Contamination**
*Contaminated plate* (growth on all agars)
Gram negative organisms cannot be differentiated at the genus level (such as E. coli, Klebsiella or Enterobacter) on the agar plates used in on-farm cultures. However, they can be identified as lactose negative or lactose positive by what color they ferment lactose in MacConkey agar. Gram negative infections often resolve on their own. Therefore, it is not always necessary to treat with antibiotics. Remember, it is always advisable to consult your local veterinarian when making these decisions.

Biplate
Gram-Negative bacteria (growth only on MacConkey and blood agars)

Triplate
Lactose-positive (pink growth on MacConkey agar) Lactose-negative (white/yellow growth on MacConkey agar)
E. coli
Klebsiella
Enterobacter

Quadplate
Gram-Negative bacteria (growth only on MacConkey and blood agars)

Contamination
Contaminated plate (growth on all agars)
Contamination

One problem that can easily occur for on-farm culture labs is contamination during sample collection, handling or plating. Contamination can be difficult to detect on selective media, since many contaminants are not able of grow. Prevention is key in reducing the number of plates that become contaminated.

Contamination during sample collection may occur if udders are not properly disinfected prior to sampling, if the teat, the cow’s tail, or another source of manure contacts the sample vial, or if the vial is not closed promptly after sampling.

Contamination during sample handling can occur if the sample is not placed in a cooler during transport to the lab, or if it is allowed to sit out for greater than one hour prior to plating.

Contamination during plating can happen if staff do not wear clean disposable gloves, if sterile swabs are left uncovered or contact non-sterile material, or if plates are not covered immediately after plating.

*Benchmark:* If more than 5% of the culture plates are contaminated, procedures should be evaluated for aseptic technique, and the appropriate changes or training should be performed.

Failure of Quality Control

Another problem that can influence the value of culture results is failure of quality control lab processes.

Incubator temperature should be maintained at 37 degrees Celsius or 98.6 degrees Fahrenheit. If the incubator temperature is too low or too high, disease-causing bacteria will not grow as well. This can lead to no-growth samples or mis-diagnoses.

Incubator humidity must remain high as well. A container of water should be kept in the incubator and refilled often to maintain moisture levels conducive to bacterial growth.

*Benchmark:* On-farm culture results should be compared to milk quality lab results at specified intervals to evaluate the quality of on-farm interpretation. They will not be identical but they should agree in general about 80% of the time.
What Can Go Wrong?

Over-interprettation of Bacterial Growth
In real diagnostic labs about 25-40% of milk samples from cows with clinical mastitis result in no-growth or non-significant growth. It is common for a few bacteria to get picked up from teat skin or during sampling, but bacteria on contaminated plates and non-significant growth are not considered the cause of mastitis. Antibiotics should not be given based on contaminated growth or non-significant growth.

Benchmark: Only when you have 1 to 2 types of colonies with at least 3-5 colony-forming-units can you be confident in your bacterial identification and choice of treatment.

Failure to Use Information Properly
The most common problem at on-farm culture labs is that farmers do not use the culture results for making treatment decisions. The value of any diagnostic test is based on the economic value of the intervention that one makes. If the culture results are not taken into account during real-time decision making, the lab is not having the impact it should on the farm, antibiotic use and costs will not be reduced, and, therefore, the value of culturing is lost.

Benchmark: Work with your veterinarian to design and implement appropriate treatment protocols for your farm. Remember, your local veterinarian and milk quality lab are here to help you. Be sure to contact them for support and accountability in maintaining the quality of your on-farm culture system.