USING ON FARM CULTURING TO IMPROVE MASTITIS TREATMENT

How to get started, collect sterile milk samples, culture bacteria and diagnose results

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ON FARM CULTURING

CLINICAL MASTITIS TREATMENT PROTOCOL

Large herd management protocol for detection and initial decision making for treatment of clinical mastitis

STEP 1. DETECTION OF CLINICAL CASE

By milking technician in parlor

Actions

1. Collect milk sample
2. Discard abnormal milk
3. Send cow to hospital after milking

Milk sample cultured either:

1. On-farm
2. Off-farm

STEP 2. ASSIGN SEVERITY SCORE

By trained hospital pen manager

Severe Case

Symptoms extend beyond udder

Non-Severe Case

Symptoms are restricted to milk and udder

STEP 3. INITIAL ACTION

By trained hospital pen manager

Immediate symptomatic treatment

Review medical history of cow

STEP 4. INITIAL DECISION FOR NON-SEVERE CASES

By hospital pen manager at admission to hospital pen before results of culture are known

Antibiotic therapy is not likely to be of benefit

Possible benefit of antibiotic therapy

STEP 5. CLINICAL CASE MANAGEMENT

By trained hospital pen worker following protocol supervised by attending veterinarian who is responsible for authorizing use of prescription and extralabel drugs.

Select a non-antibiotic case management option

Culture-Based Therapy

Option 1: Delayed RX

Option 2: Immediate RX

Option 3: Symptomatic intramammary RX for 1-3 days

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NON-SEVERE CLINICAL MASTITIS TREATMENT THERAPY

Considerations for use of intramammary (IMM) antibiotics for treatment of non-severe clinical mastitis (severity scores 1 & 2) occurring in cows with a medical history that indicates antibiotic therapy may be justifiable but culture based therapy is not possible.

1st choice 2nd choice 3rd choice

Medical History of Cow		Priority of Treatment Choices		
		Watchful Waiting (no IMM antibiotic usage)	Short Duration (1-3 days) IMM Treatment	Long Duration (4-8 days) IMM Treatment
Lactation number	1-2	2nd choice	1st choice	3rd choice
	≥3	3rd choice	1st choice	2nd choice
Number of monthly SCC >200,00 cells/mL	≤2	2nd choice	1st choice	3rd choice
	3+	3rd choice	2nd choice	1st choice
Stage of lactation (DIM)	≤60	3rd choice	1st choice	2nd choice
	>60	2nd choice	1st choice	3rd choice
Previous treatment for clinical mastitis in same 1/4	No	2nd choice	1st choice	3rd choice
	Yes	3rd choice	2nd choice	1st choice

MASTITIS TREATMENT PROTOCOL

Milker Detects Clinical Mastitis

Severity Score is Assigned by Milker

Mild (abnormal milk)

Moderate (abnormal milk + abnormal quarter)

Severe (systemic signs) → **Do Not Culture:** Treat immediately according to protocol designed by attending veterinarian

Set up On-Farm Culture, Send Cow to Hospital Pen and Discard Milk

Immediate Rx:

Start Rx upon case detection and adjust therapy 24 hrs later based on culture result

Staph. aureus → Check SCC

- **New Infection:** Single quarter, 1st or 2nd lactation → consider therapy but expect only 50-60% success
- **Chronic Infection:** Stop Rx, segregate and confirm culture in lab. If *S.aureus* → Cull

Gram-positive (NOT *Staph. aureus*)

- ***Strep***: Continue Rx for defined duration (short or extended depending on medical history)
- ***CNS***: Stop therapy after short duration therapy is completed

Gram-negative → Stop Rx or if indicated change to a Gram-negative spectrum IMM drug for short duration

No Growth → Stop Rx, milk will usually return to normal in 4-6 days

Delayed Rx:

Wait 24 hrs for diagnosis and start Rx based on culture result

Staph. aureus → Check SCC

- **New Infection:** Single quarter, 1st or 2nd lactation → consider therapy but expect only 50-60% success
- **Chronic Infection:** Stop Rx, segregate and confirm culture in lab. If *S.aureus* → Cull

Gram-positive (NOT *Staph. aureus*)

- ***Strep***: Continue Rx for defined duration (short or extended depending on medical history)
- ***CNS***: Begin short duration antibiotic therapy

Gram-negative → Do not treat with antibiotic (or if medical history indicates use IMM Gram-negative spectrum drug)

No Growth → Do not treat with IMM unless medical history indicates therapy may be needed

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CLINICAL MASTITIS TREATMENT PROTOCOL

Milker detects clinical mastitis. Severity score is assigned.

NON-SEVERE CLINICAL CASE

Set up on-farm culture.
Send cow to hospital pen and discard milk.

SEVERE CLINICAL CASE

Do not culture. Treat immediately according to protocol designed by attending veterinarian.

	DELAYED TREATMENT Wait 24 hours for culture results before starting antibiotic therapy.	IMMEDIATE TREATMENT Start short duration intramammary treatment immediately and adjust treatment plan after 24 hours based on culture results.
	CULTURE RESULTS AT 24 HOURS	
No Growth or Non-Significant Growth	Do not administer intramammary antibiotic unless medical history of cow indicates that her immune system is compromised. Discard milk until it returns to normal (usually 4-6 days).	Stop treatment. Discard milk until it returns to normal and the antibiotic withholding period has ended.
Gram-Negative Growth	Do not administer intramammary antibiotic unless medical history of cow indicates that her immune system is compromised. If intramammary antibiotic is used, ensure that it has a Gram-negative spectrum.	Stop treatment or if medical history indicates antibiotic therapy may be useful, change to an intramammary drug that has Gram-negative spectrum for short duration.
Gram Positive Growth Non-Specified Organisms	Give intramammary treatment for 1-3 days using narrow sprectrum antibiotc.	Stop therapy after completion of 1-3 days of intramammary treatment using narrow spectrum antibiotic.
Gram Positive Growth Likely CNS	Give intramammary treatment for 1-3 days using narrow sprectrum antibiotc.	Stop therapy after completion of 1-3 days of intramammary treatment using narrow spectrum antibiotic.
Gram Positive Growth Likely *Streptococci spp.*	Give intramammary treatment short or long duration depending on medical history of cow.	Discontinue intramammary treatment if short duration is appropriate. Continue intramammary treatment if medical history of cow indicates longer duration therapy is justifiable.
Gram Positive Growth Likely *Staph. aureus*	Review medical history of cow before considering treatment. Segregate cow after treatment is completed. Do not treat cows with history of chronic clinical or subclinical infections.	

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ON FARM CULTURE LABORATORY SET UP

On farm culture systems require a **designated workspace**. Ideally, this should be a clean, well-lit room in a low-traffic area. There should be sufficient counter space and storage that are easily disinfected. Food should not be allowed in this area for health reasons.

CHECKLIST

Incubator

- o A small **incubator** should be purchased to create an environment ideal for bacterial growth.
- o Keep the incubator at 37*C or 98.6*F (body temperature).
- o A thermometer should be kept inside the incubator at all times, and should be checked daily.
- o The humidity should be maintained at 75% by placing a dish of water inside the incubator. Water level should be checked daily and replenished as needed.

Refrigerator

- o A **refrigerator** should be purchased for storing media plates and saved milk samples. This refrigerator should not be used for human or animal food.

Equipment

- o On-farm cultures use **sterile swabs** to plate milk samples instead of the sterile loops used in milk quality labs. The estimated plating volume of swabs is 0.1 mL if the swab is dipped in the milk sample for 10 seconds prior to plating.
- o **Disposable gloves** should be worn at all times when handling lab materials.
- o **Media plates**
- o **Gauze squares** soaked in **70% alcohol** for disinfecting teats and the counter surface
- o **Single-use milk sample vials**
- o **Racks for holding sample vials**
- o **Permanent marker**
- o **Biohazard bags**
- o **Bleach**
- o A **cooler** with ice for transporting samples from the cow to the lab area.

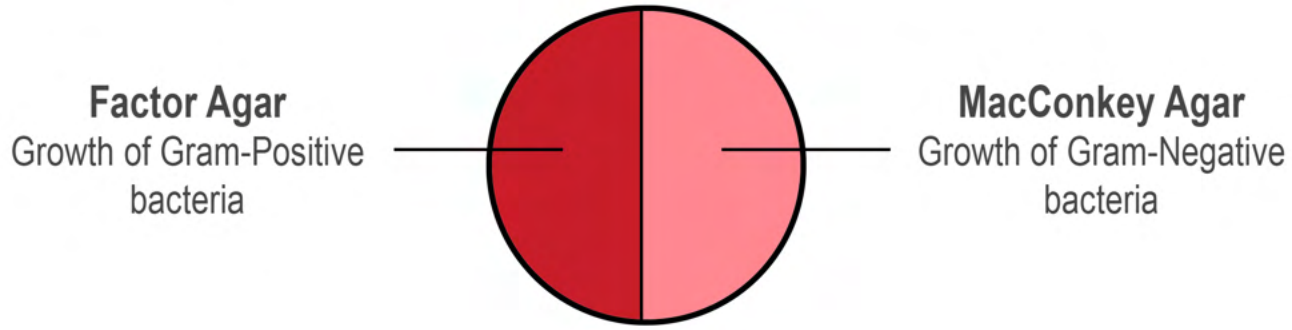
Plate Disposal

- o One more consideration for lab set-up is waste disposal. Use orange biohazard bags whenever discarding infectious materials such as milk samples and culture plates.
- o This lab waste must be disposed according to your local regulations. In some locations, plates may be flooded with bleach and then disposed of normally
- o If you have questions about disposal protocol in your area, ask your herd veterinarian.

SELECTING CULTURE MEDIA

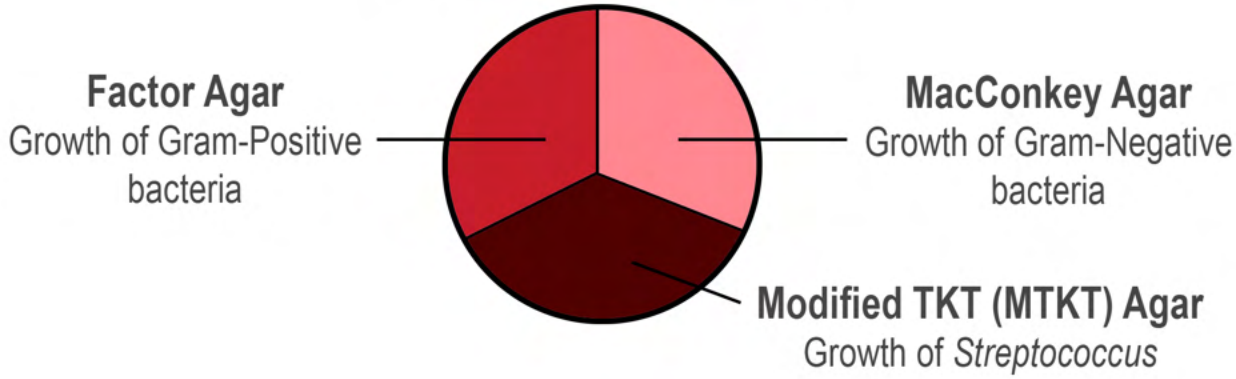
Biplate

Results: Gram-positive, Gram-negative bacteria, No growth or contaminated



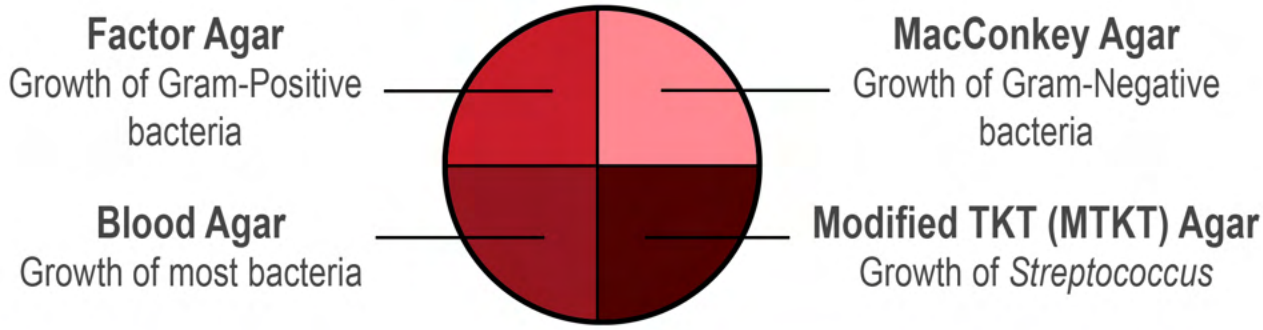
Triplate

Results: Staphylococcus spp., Streptococcus spp., Gram-negative bacteria, No growth or contaminated



Quad plate

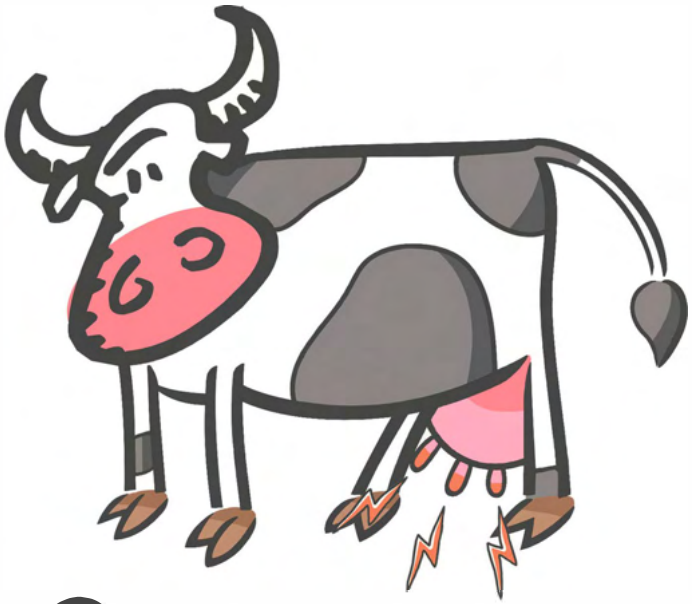
Results: Staphylococcus spp., Streptococcus spp., Gram-negative bacteria, No growth, contaminated or others.



CLINICAL MASTITIS SEVERITY SCORING GUIDE

Clinical mastitis is an udder infection that shows symptoms which are visible. The level of infection, or severity, can help herd managers make treatment decisions. The degree of illness and the symptoms present will depend on many factors, such as the nutritional or immune status of the cow, which pathogen is responsible for the inflammation, and a range of environmental factors such as cleanliness, humidity and ambient temperature. Moderate to severe clinical cases can be very painful and unpleasant for the cow.

1 ABNORMAL MILK
Milk has a watery appearance, flakes or clots.



2 ABNORMAL UDDER
Signs of inflammation: swelling, heat, hardness, redness or pain.

3 ABNORMAL BEHAVIOR
Reduction in milk, fever, lack of appetite, sunken eyes, diarrhea, dehydration or reduction in mobility.

1 = MILD

1 + 2 = MODERATE

1 + 2 + 3 = SEVERE

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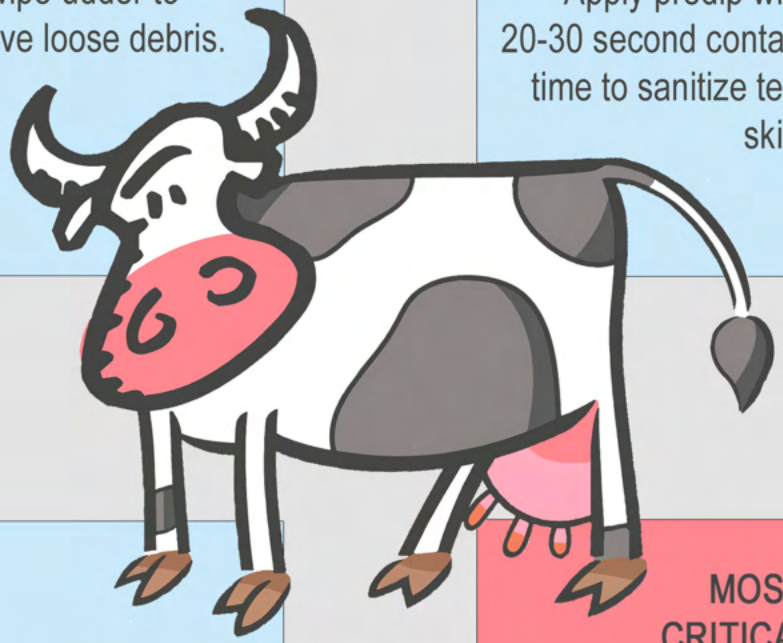
HOW TO COLLECT A STERILE MILK SAMPLE

The entire process of performing on farm culturing is absolutely dependent on how well the milk sample was collected. If this step is not performed correctly, then the rest of the process will be meaningless. Follow these steps to help collect a sterile milk sample.

1. After detecting the mastitis case, label a sterile (unopened) vial with the cow ID, quarter and date.
2. Dry wipe udder to remove loose debris.
3. Apply predip with 20-30 second contact time to sanitize teat skin.
4. Dry off teat with single-use towel.
5. Forestrip 3-4 streams of milk to remove bacteria from teat canal.
6. **MOST CRITICAL** Scrub end of teat with gauze swab soaked in 70% alcohol.
7. Collect milk sample into the labeled vial.
8. Immediately cool sample in a cooler or refrigerator until plating can occur.



HOW TO COLLECT A STERILE MILK SAMPLE

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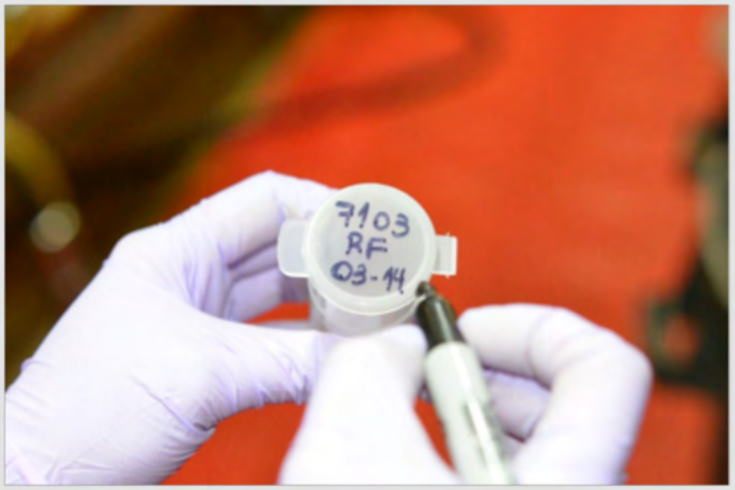
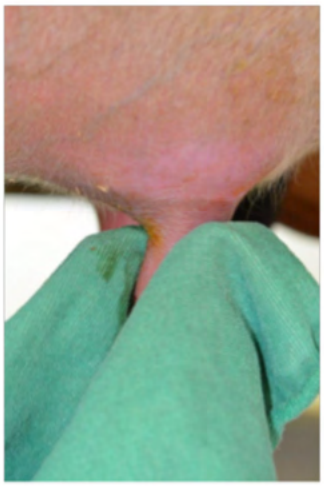
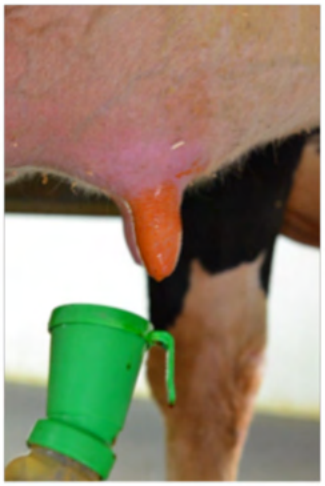


2. Dry off teat with single-use towel.

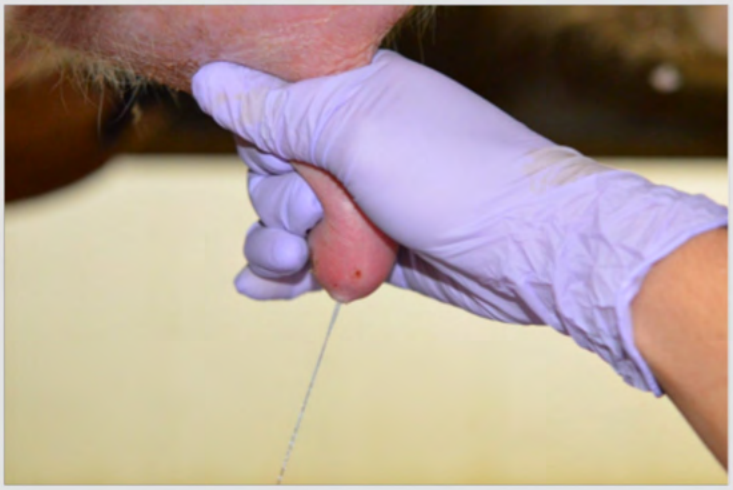
3. Apply predip with 20-30 second contact time to sanitize teat skin.





4. Dry off teat with single-use towel.

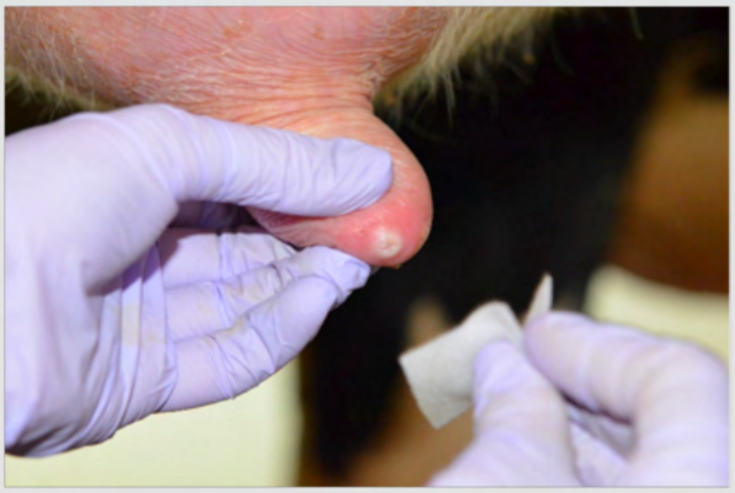
5. Forestrip 3-4 streams of milk to remove bacteria from teat canal.



HOW TO COLLECT A STERILE MILK SAMPLE

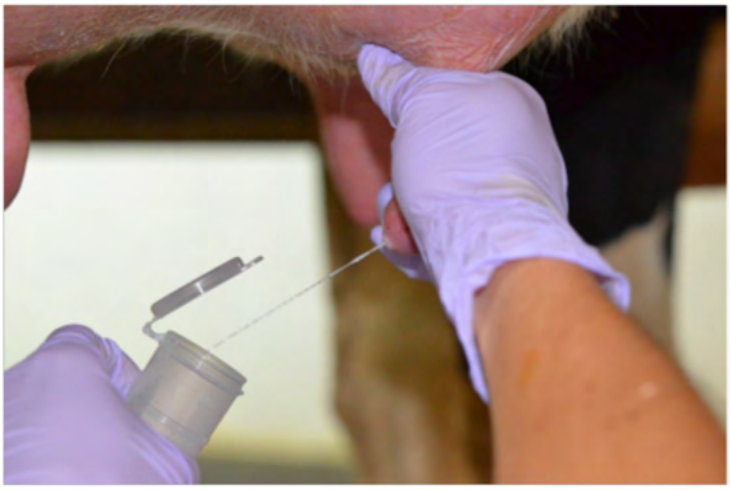
6

MOST CRITICAL
Scrub end of teat with gauze swab soaked in 70% alcohol.



7

Collect milk sample into the labeled vial. Do not touch any inner part of the vial to avoid contamination of the sample.



8

Immediately cool sample in a cooler or refrigerator until plating can occur.

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.

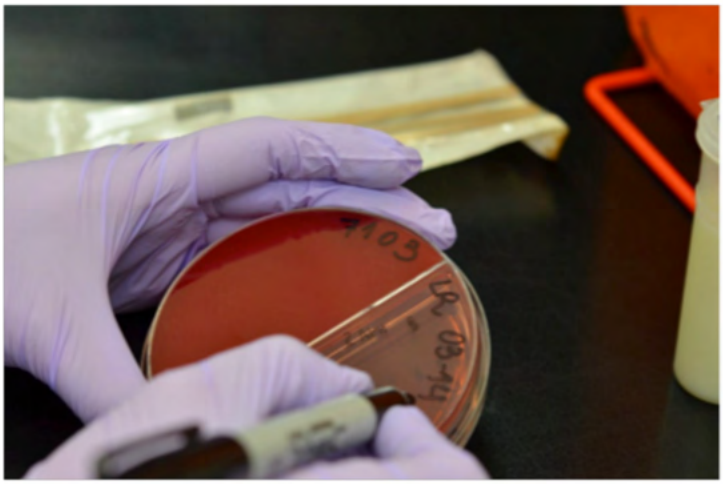
Place the upside-down plates in the incubator and incubate at 37 degrees Celsius (98 degrees Fahrenheit) for 24 hours. The bacteria will need a minimum of 24 hours to grow before reading results.

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

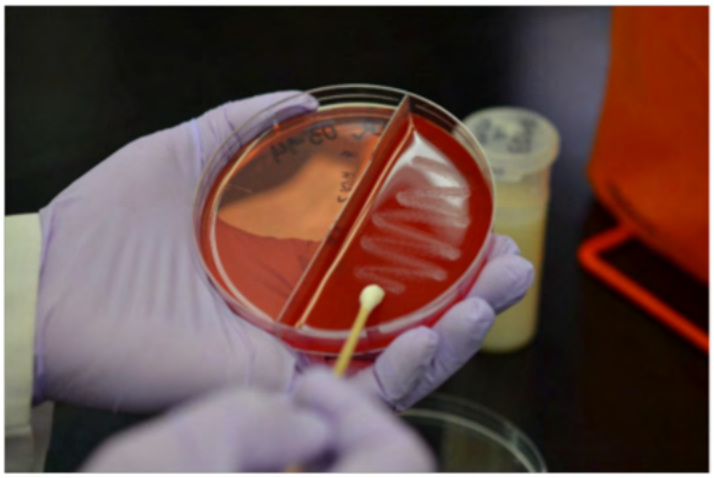


Thoroughly mix the milk sample by inverting it several times.

HOW TO SET UP CULTURE PLATES

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

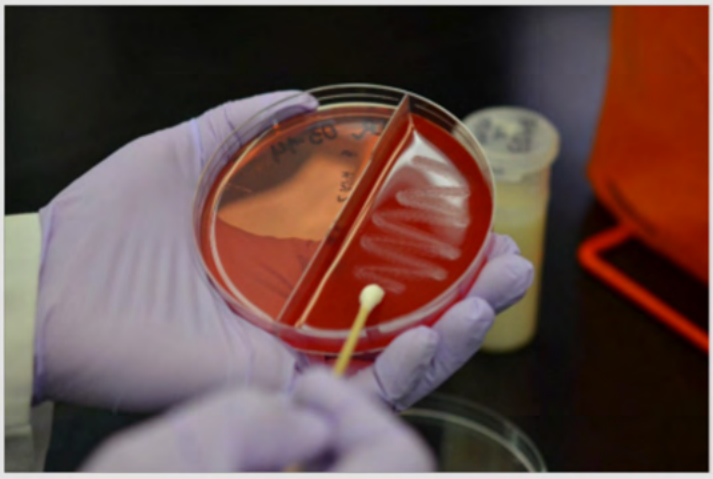
Inoculate one section of a culture plate using a zigzag streaking pattern.



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

HOW TO SET UP CULTURE PLATES

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.

HOW TO READ CULTURE PLATES

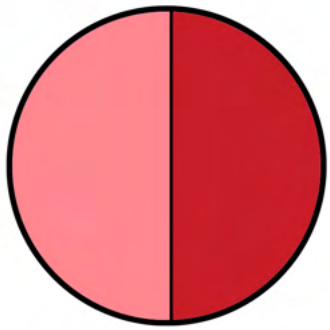
The purpose of performing on farm culturing is not only to grow bacteria but to ensure that our diagnosis is correct so that we can determine if antibiotic treatment will be helpful.

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?**

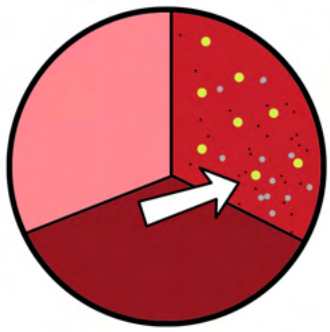
No Growth

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.



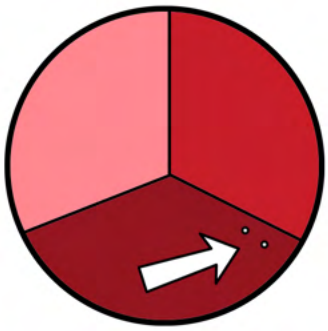
Second Step: Interpretation of Bacterial Growth

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



Contaminated

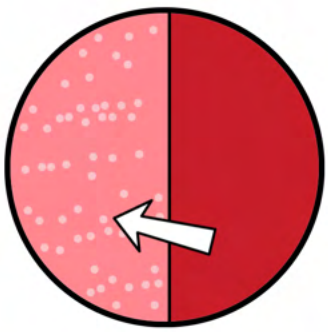
If there are more than 2 types of colonies, the plate has been contaminated. When this happens, the quarter may be resampled using proper collection technique and cultured again.



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.

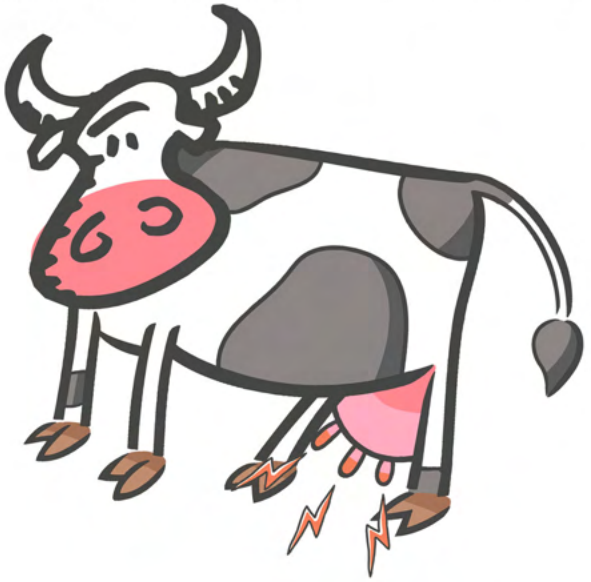


True Infection

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.

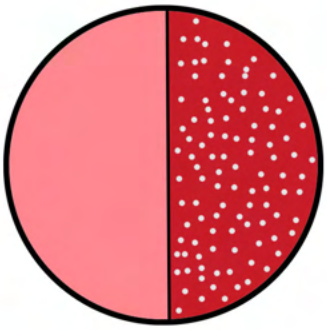
HOW TO IDENTIFY STAPHYLOCOCCUS SPECIES

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.



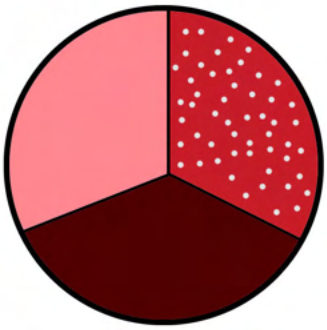
Biplate

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

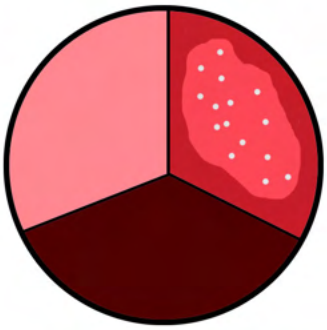


Triplate

Staphylococci (growth only on Factor or Blood agar)

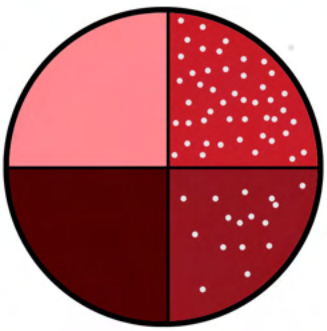


Staphylococcus aureus (zone of hemolysis)



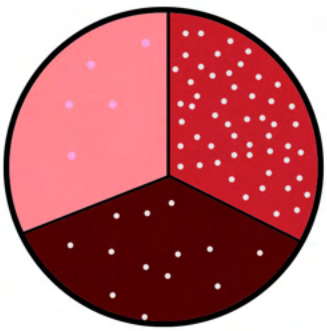
Quadplate

Staphylococci (growth on Blood Agar and Factor agar)



Contamination

Contaminated plate (growth on all agars)

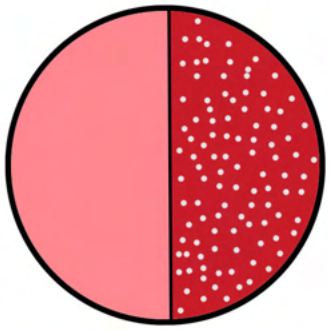


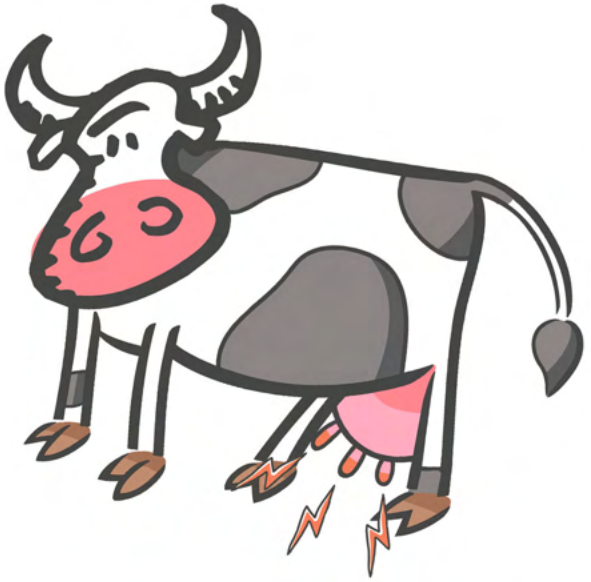
HOW TO IDENTIFY STREPTOCOCCUS SPECIES

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

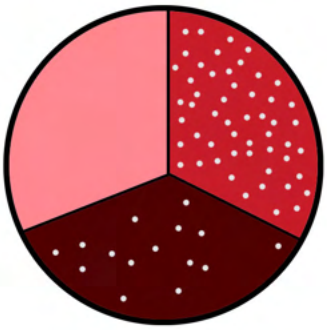
Gram-Positive bacteria (growth only on Factor or Blood agar)



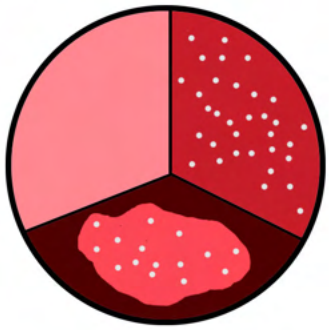


Triplate

Streptococci (growth on both Factor and MTKT agars)

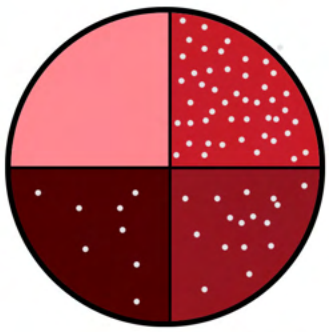


Streptococcus agalactiae (zone of hemolysis in MTKT)



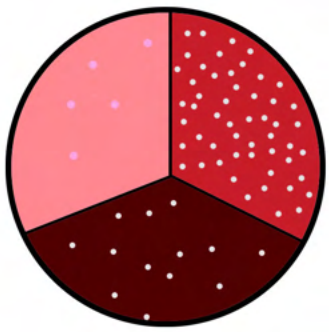
Quadplate

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



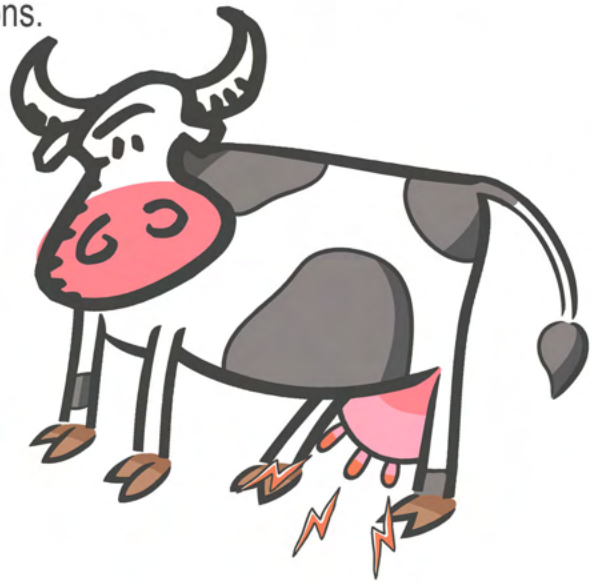
Contamination

Contaminated plate (growth on all agars)



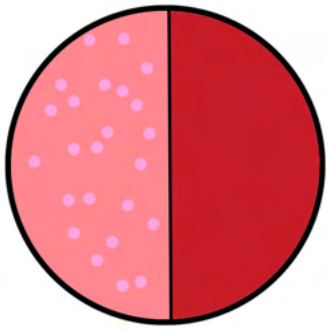
HOW TO IDENTIFY GRAM NEGATIVE SPECIES

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.



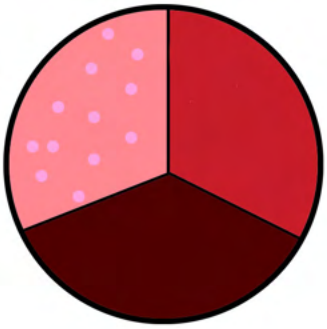
Biplate

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

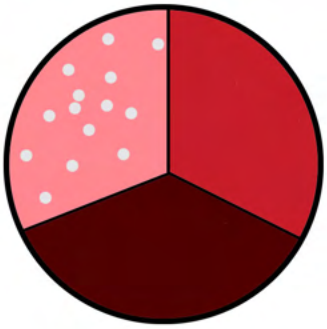


Triplate

Lactose-postive (pink growth on MacConkey agar)
E. coli
Klebsiella

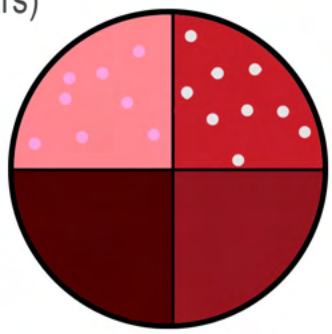


Lactose-negative (white/yellow growth on MacConkey agar)
Enterobacter



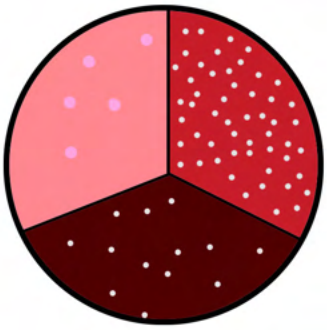
Quadplate

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



Contamination

Contaminated plate (growth on all agars)



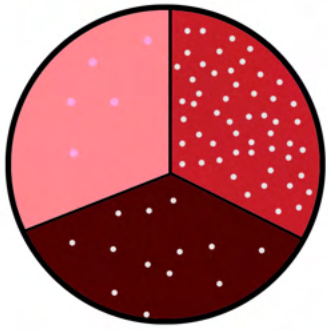
WHAT CAN GO WRONG?

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

Contaminated plate (growth on all agars)



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?

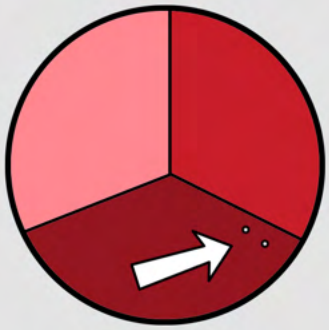
Over-interpretation of Bacterial Growth

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.

Non-significant Growth

Fewer than 3-5 cfu's per colony type



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.