On-Farm Mastitis Bacteria Identification Tool

Mastitis Treatment Decision System

Réseau canadien de recherche sur la mammite bovine
Canadian Bovine Mastitis Research Network

Why is this tool valuable?

Mastitis is the most frequent cause of antibiotic use on dairy farms. Various bacteria can cause udder infections, some of which have higher rates of self-curing or respond poorly to treatment. Correct identification is crucial to allow dairy producers and veterinarians to select, if needed, targeted treatments without compromising or delaying recovery.

Gram + and Gram – Bacteria

In laboratory, staining procedures are used to classify most bacteria into two categories:

Gram positive bacteria

- Mainly staphylococci and streptococci
- Thin cell walls

Gram negative bacteria

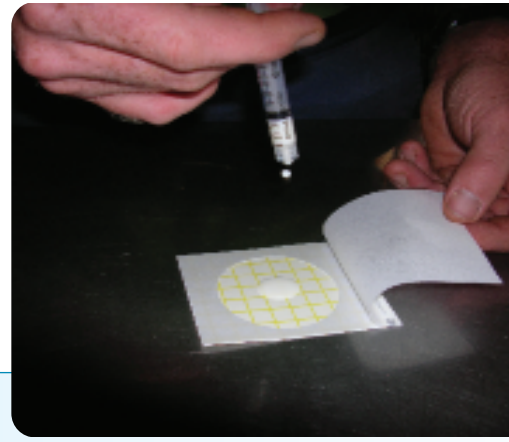
- Mainly coliforms
- Thick cell walls which protect against the effects of most antibiotics, detergents and chemicals
- Contain toxic lipopolysaccharides
- Do not respond well to antimicrobial treatment

Examples of bacteria Gram + and Gram -

Gram positive	Gram negative
Staphylococcus aureus	*Escherichia coli*
Staphylococcus spp.	*Klebsiella* spp.
Streptococcus uberis	*Enterobacter* spp.
Streptococcus dysgalactiae	*Serratia* spp.
Streptococcus agalactiae	*Pasteurella* spp.
Corynebacterium bovis	

How does it work?

Bacteria are identified through milk culturing on plates containing nutrients that promote bacterial growth. An approximately 24-hour incubation period is required for the presence of bacterial colonies to be revealed. Various plates with specific nutrients can be used to select for appropriate bacteria present in milk. The category of bacteria is based on growth and colony numbers on specific plates.



Advantages

- Allows identification and targeting of cases caused by Gram + bacteria (requiring treatment)
- Quicker identification of bacteria compared to bacterial cultures in laboratory (not to replace it)
- More rational use of antibiotics
- Decreased milk losses
- Decreased risk of antibiotic residues

Limitations

- Care required in technique to avoid contamination
- Interpretation influenced by the experience and training of the user
- Cannot identify mycoplasms and yeasts (treatment not recommended)

01/09/10

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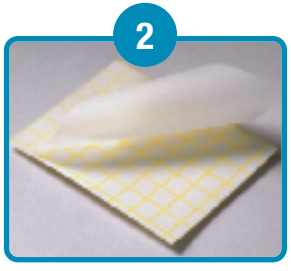
CAUTION

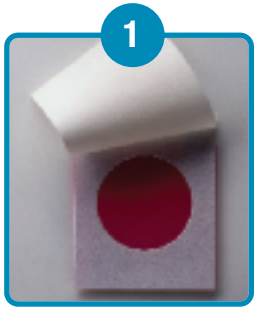
- The on-farm identification system should be used in mild or moderate cases of mastitis (grade 1 or 2) without fever (< 39.5 °C) and in the absence of other severe diseases.
- It is critical to develop a sampling protocol and a treatment protocol based on the use of this system in collaboration with your veterinarian.
- If clinical symptoms worsen, take immediate action and consult your veterinarian.

Necessary Materials

Included in Maritime Quality Milk (MQM) kit:

- Petrifilm™ Coliform Count Plates (GRAM –) red circle with polystyrene foam, marked CC (1)
- Petrifilm™ Aerobic Count Plates (GRAM +) white without polystyrene foam, marked AC (2)
- Spreader (ridged and flat sides)
- 3 ml syringes
- Sterile sampling vials
- Vials of diluent (0.9% NaCl saline or PBS)
- Alcohol swabs





Not included in MQM kit:

- Incubator*
- Nitrile gloves
- Petrifilm™ Staph Express (staphylococci) Count Plates: white circle with polystyrene foam, marked STX and purple Staph Express disk (optional for *Staph. aureus* detection) (3)
- Spreader for Staph Express (round and flat)

At the farm, home or veterinary clinic, set up the "laboratory" in a clean and dry area to avoid contamination.

*The recommended incubator is the Turbofan Hova-Bator 1602, available from your local agricultural retailer; consult internet to locate the dealer nearest you.

Storage and Handling of Plates of MQM Kit

- Can be stored for 1 year at room temperature
- Avoid parlours and dairies (high humidity)
- Keep the plates in their original packaging until use
- Do not expose to sunlight and discard any discoloured plates
- Do not touch plate surface

Plates not sold as part of MQM kit and not sealed, should be kept frozen.

The MQM Mastitis Treatment Decision System uses 3M™ Petrifilm™ enumeration plates to identify mastitis bacteria in milk. To order an on-farm MQM kit, please contact your local veterinarian or:

Maritime Quality Milk

550 University avenue, Charlottetown,
Prince Edward Island, Canada C1A 4P3
Phone: 902-566-6489 / Fax: 902-620-5053
Email: mqm@upei.ca
www.milkquality.ca

To order Petrifilm™ products directly or to consult a representative, please contact:

3M Canada

Phone: 1-800-563-2921

Source and Photo credits :
Maritime Quality Milk (MQM), Charlottetown, PEI
3M Canada

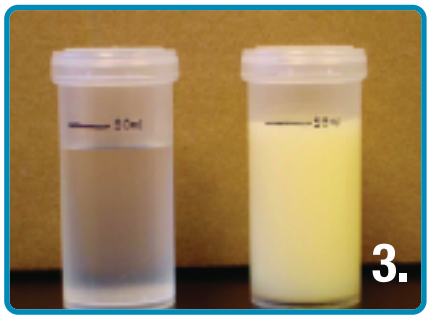
Inoculation of the AC and CC Petrifilm™ Plates

Materials

Prepare all the necessary materials before beginning.

Milk sampling

- The quality of the sample is critical to good results. Use a clean sampling vial.
- To avoid sample contamination and incorrect interpretation, the sampling process must be sterile.
- Consult the CBMRN's "Milk Sampling Collection Technique for Bacterial Testing" practical sheet or "ABC of Milk Sampling for Bacterial Testing" video for assistance. **www.mastitisnetwork.org**



Milk dilution

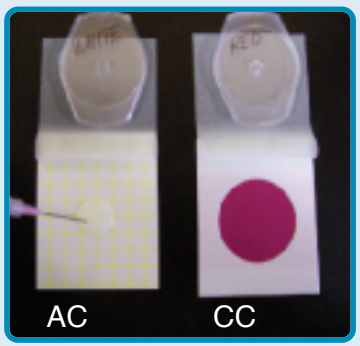
Open the vial containing 45 ml of water and add approximately 5 ml of infected milk, to 50 ml. Recap both vials. Clearly label the vial containing the unused infected milk and freeze for shimpment to the laboratory, if necessary.

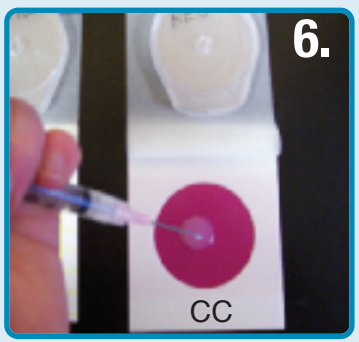
Mixture preparation

Gently rotate the vial to mix the sample.

Measuring diluted milk

Draw 2 ml of diluted milk with the syringe.

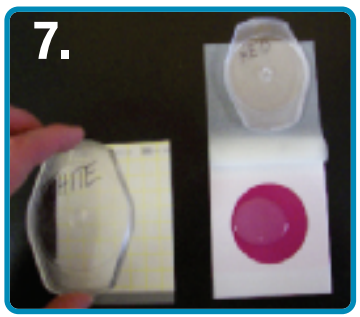




Inoculation of the plates

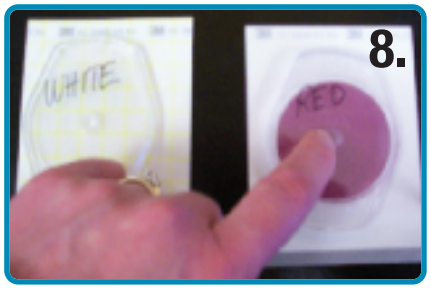
Lift the film and deposit 1 ml of diluted milk directly in the centre of each plate without touching it with the syringe.

For CC plate, slowly roll the film onto the milk. For AC plate, drop the film onto the milk without rolling. For all plates do not touch the plate surface.



Milk spreading on White AC Plate

Carefully deposit the ridged side of the spreader (WHITE side up if you use MQM kit) on the film and press lightly to spread the milk.



Milk spreading on Red CC Plate

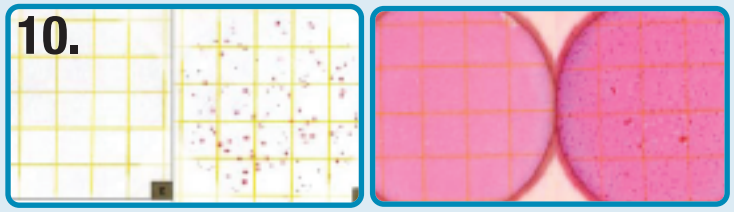
Carefully deposit the flat side of the spreader (RED side up if you use MQM kit) on the film and press lightly to spread the milk.

Placing plates in the incubator

Carefully place the plates in the incubator pre-heated to 35 °C. Ensure the incubator contains a little bit of water to maintain slightly humid conditions.

Incubation time for White AC and Red CC Plates

The required incubation periods are as follows:



- 24 hours for the aerobic bacteria enumeration plate
- 24 hours for the coliform enumeration plate

Interpretation of the Results

- Your veterinarian should be engaged to develop treatment protocols for your herd according to results.
- Categorization into *Gram positive*, *Gram negative* and *No growth* can be made with the AC and CC plates. Interpretation guidelines are available at **www.milkquality.ca**
- For disk confirmed Staph Express positive cultures, treat these cases as advised by your veterinarian.

Inoculation of the STX Petrifilm™ Plates

1. Inoculation

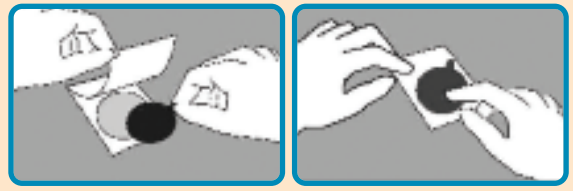
Lift the film and deposit 1 ml of diluted milk perpendicularly and directly in the centre of each plate without touching it with the syringe. Slowly unroll the film onto the milk.

2. Spreading milk

Carefully deposit the round and flat spreader on the film and press lightly to spread the milk.

3. Incubation time

Incubate at 35 °C the STX plate for 24 hours.



4. Insertion of the purple disk

Insert the purple disk between the film and the plate. Incubate 3 more hours at 35 °C following the addition of the disk.

Proceed to interpretation.