A Milk cultures

What is milk culturing?

It is not possible to tell which bacteria are responsible for infections by looking at milk, udders or somatic cell counts- you have to actually grow the bacteria to know for sure.

A milk sample can be processed in a microbiology laboratory so that the bacteria that are present can be identified. This involves the laboratory spreading some of the milk on sterile plates covered with particular growth factors, incubating the plates for defined periods and assessing the bacterial colonies that grow. This is called milk culturing.

The laboratory can also check if the bacteria are resistant or sensitive to a predetermined list of antibiotics (called 'sensitivity testing'). However, these tests only provide a guide as conditions on the laboratory plate are not always exactly the same as in the cow's udder. Other factors such as duration of infection, lactation number etc. will also influence treatment outcomes.

PCR tests are now available. These detect the presence of bacterial DNA and therefore, do not rely on the viability of bacteria for results. Sometimes milk culture samples will yield no significant growth which can be frustrating. The most common reason is a decline in the number of live bacteria in the sample or where the sample contains a mixture of contaminants due to a poorly taken sample. PCR testing may reduce the incidence of 'no growths'.

When is it worthwhile culturing milk samples from cows?

Milk cultures are recommended whenever a herd problem emerges- either more clinical cases than acceptable, or rising somatic cell counts. They are used to indicate which bacteria are present within the herd, so samples from a number of cows (a minimum of 10) are required to give a representative picture.

Virtually all mastitis is caused by bacterial infection. It is essential to know which bacteria (e.g. *Staph. aureus*, *Strep. agalactiae*, *Strep. uberis*) are present to decide where to look for problems and select appropriate management strategies.

Cost is important but if a herd mastitis problem is emerging, the cost of the cultures is likely to only be a small issue. The cost varies depending on factors such as the number submitted at the same time and transport costs etc. PCR is more expensive but results will be available quicker and the tests have an increased sensitivity.

Is it important to sample quarters with clinical mastitis?

Yes, in most cases it is a good insurance policy to take samples- but you will not necessarily choose to have them all cultured. You can collect them from all clinical cases before treatment (any antibiotic in the sample will make it very difficult to culture), store them frozen and only submit them if a cow fails to respond to treatment, or if there are a higher number of cases than you expected (e.g. more than five clinical cases per 100 cows in the first month of lactation).

If you only end up having a couple of cases and they respond to treatment, you do not need to send the samples for culture. However, if you do wish to investigate further, you have valuable material to start with.

What about cows with high cell counts?

To investigate a herd cell count problem, it is sensible to sample some of the individual cows with high cell counts. Here the sample is often a composite one (some milk from all four quarters) and some infections will be missed because the bacteria from an infected quarter are diluted by milk from the other quarters. To avoid this, use your CMT to detect which quarter is infected and then sample from this quarter. To ensure you get results from at least 10 cows, it is worth taking samples from at least 12 - 14 cows. The ideal is to sample 10% of the highest cell count cows ensuring a mixture of age and infection duration. Talk with your CellCheck advisor about which are the best cows to include, to get the most informative results.

Are there any traps in milk culturing?

The main problems are associated with collection and transport of samples. Milk can become contaminated with bacteria during or after collection. Most of these bacteria can also cause mastitis, so results can be very confusing (and difficult to interpret) if any contamination occurs even with PCR testing. Gloves and disinfectant should be used when collecting milk samples, both to avoid contamination and to limit any spread of bacteria between cows.

There is a specific procedure to follow to collect good quality milk samples. Bottles must be sterile (not just clean) and teat ends must be scrubbed with 70% alcohol or methylated spirits to disinfect them adequately. You can get sterile containers from veterinary clinic, laboratory or milk processor. The step-by-step guide below tells you how to collect the milk.

Ideally samples should be kept cool and arrive at the laboratory within 24 hours. If this is not possible, most mastitis bacteria survive freezing, so you can store them in the freezer until delivery. Samples can be stored by freezing for up to four months without any negative effect on most major mastitis pathogens.

Collecting milk samples

Sterile collection is the most important step for successful culturing of milk samples. Poor technique will give misleading results and resampling will be required. A good technique involves planning and some patience.

Have the following ready in the dairy

- Sterile sample bottles. You can obtain these from your veterinary clinic, laboratory or milk processor. Sterile collection requires using only sterile bottles.
- A marker to label the bottle.
- Disposable gloves.
- Disinfectant/cleaning solution.
- Paper towels.
- Cotton balls or medicated wipes.
- A mixture of 70% alcohol or disinfecting teat wipes.
- A cool dry place to store samples before delivery or freezing.

Medicated teat wipes are excellent for disinfecting teat ends.

Label the bottle

- Unlabelled samples are useless, so make sure you identify the sample.
- Do this before sample collection as it can be difficult to write on a label with milk sprayed on it.
- Use the marker pen to clearly label details of the sample - the date, your name, the cow's ID, the quarter sampled, and why the sample was collected i.e. clinical case, high SCC etc.

Correctly labelled and sealed bottles are essential.

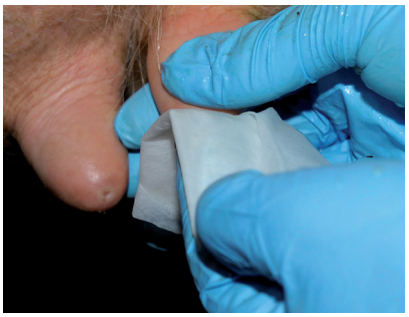
Restrain the cow so she cannot move around too much

- Sometimes this is difficult on a platform, but do what is possible.
- Another person holding the cow's tail as a 'tail jack' can be helpful.

Put on disposable gloves

Wash and dry the teats

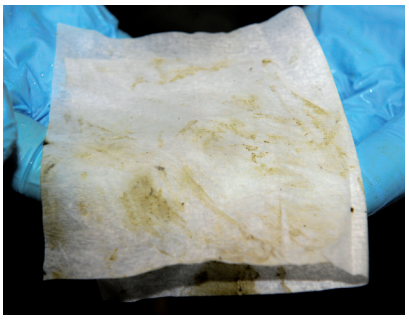
- Wash the teats with running water and disinfectant/ cleaning solution. Avoid getting water on the udder - the udder is hard to dry, and drops of contaminated water can easily fall in the sample.
- Dry with a paper towel.



Disinfect vigorously with cotton wool or teat wipes.

Completely disinfect the end of the teats to be sampled. This step is critical!

- If you are sampling more than one teat, disinfect the ones furthest away first. This reduces the risk of unintentionally contaminating an already disinfected teat.
- Disinfect by vigorously scrubbing the teat opening with a cotton ball and alcohol (or teat wipes) for a minimum of 10 seconds.
- Check the cotton ball/wipe. If it is dirty, repeat the scrub using a clean cotton ball until there is no more dirt seen.

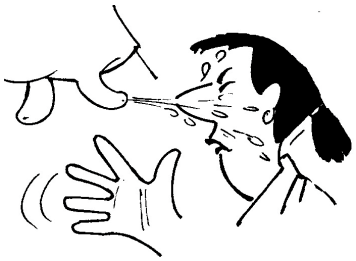


Check that the teat is fully clean.

Get the sterile bottle ready

- Remove the cap and place upside down in a place not likely to be contaminated.
- Do not touch the inside surface of the cap or bottle.

Establish the direction of flow from the teat



- Squeeze the first couple of squirts of milk onto the ground.
- This also helps to remove any contaminants that might be just inside the opening of the teat.

Collect the sample in the bottle

- Hold the bottle at an angle (to avoid anything falling into it) at least 3-4 cm from the end of the teat.
- Squirt 2- 4 mL of milk into the bottle. Only a small amount of milk is required for culture - trying to get a large sample increases the chance of contamination.
- If you are collecting a combined sample from all quarters, move the bottle away from the first teat and repeat the initial squirts of the next teat before moving the bottle back. Take the first samples from the teats closest to you. Try to get the same amount from each teat. (**Note:** a combined sample from all quarters may be less likely to grow bacteria because milk from one infected quarter is diluted by milk from the uninfected quarters.)

Replace the cap and secure it tightly and keep in a cool dry place until it can be refrigerated, delivered or frozen

- If there are any obvious contaminants or for example the lid was dropped, then take the sample again starting with sterilising the teats.

Wash your hands

- Use running water and dry on paper towel.
- Wash your hands after each cow, including the last cow.

Sample handling and storage

- Ensure that the bottle is labelled correctly with farm and animal details.
- Deliver the sample to the veterinary clinic or laboratory that day, or freeze.
- Samples for mastitis culture can be frozen and stored before being sent to the laboratory.
- Samples can be stored for up to 4 months without any negative effect on most major mastitis pathogens.

MANAGEMENT NOTES