SECTION II-5
HOW DO WE DETECT MASTITIS IN AN INDIVIDUAL SHEEP?

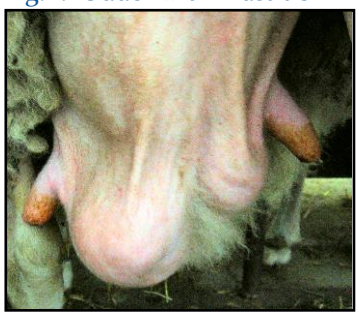
5. HOW DO WE DETECT MASTITIS IN AN INDIVIDUAL SHEEP?

5.1 EXAMINATION OF THE UDDER AND TEATS

5.1.1 UDDER

When a gland has a clinical mastitis infection, the signs are usually obvious; the affected gland or half is often enlarged and swollen. If the infection is severe enough, the udder is red in colour (indicating that it is inflamed), and is hot to the touch. Ewes are susceptible to a condition called "blue bag" in which the toxins of the bacteria (usually *Staph. aureus* but sometimes *Mannheimia* or *Pseudomonas*) cause the tissues of the gland to die and become gangrenous. The gland is cold to the touch and blue and the ewe is very ill. If the infection is chronic, the udder can be shrunken, hard and may contain lumps or abscesses (Fig. 21).

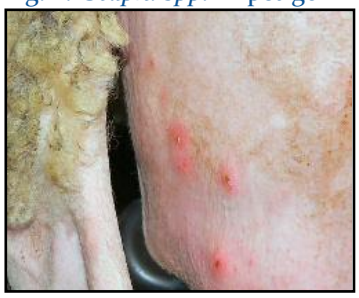
Fig. 1. Udder with mastitis



Maedi visna virus targets the udder, but symptoms are different from bacterial infections. The udder is often called "hard bag", and appears as though it is full with milk, however, it is quite hard, and very little milk can be removed from the gland.

Staphylococcal impetigo is an udder skin condition that is associated with *Staph. aureus* or other *Staph.* bacteria, which are common causes of clinical mastitis in sheep. This develops a rash with small bumps on the udder, and if not treated, can possibly allow bacteria to enter the udder through the skin (Fig. 22).

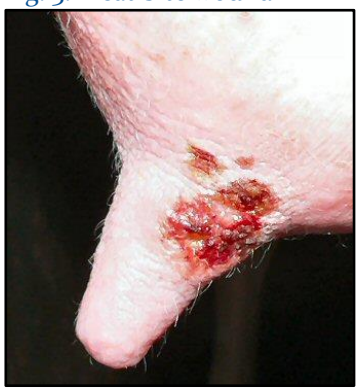
Fig. 2. *Staph. spp.* impetigo



5.1.2 TEATS

Teat end health is very important to help control udder health in sheep. The conditions of the teats are discussed in Section II.4.6 and II.4.7. Poor vacuum and equipment function during milking can cause damage to the teat end. First evidence is a raised ring around the teat canal, leading to build-up of scar tissue around the teat opening – also known as hyperkeratosis (**Error! Reference source not found.**). This condition leads to the teat end being very rough, and often harbours mastitis-causing bacteria.

Fig. 3. Teat bite wound

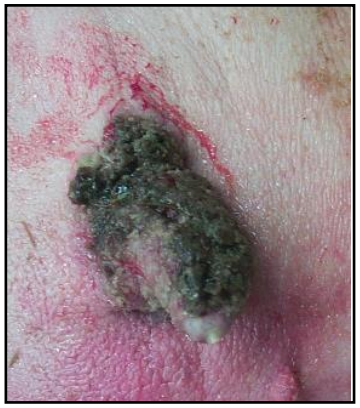


Build-up in the teat, which prevents milk from being released from the udder, is called a pea. This is fibrous tissue caused by infection or

trauma that will block milk in the teat. Sometimes it can be removed by a veterinarian but the damage may be permanent.

In ewes that are still nursing their lambs, there is a risk of teat damage due to teat biting (Fig. 23). This can cause lesions which could be very painful to the ewe during milking, and can potentially harbour contagious pathogens. Nursing ewes may develop contagious ecthyma (orf, soremouth) on the teats, picked up from the lambs (Fig. 24).

Fig. 4. Contagious ecthyma – teat Dr. M. Smith, Cornell U



5.2 EXAMINATION OF THE MILK OF AN INDIVIDUAL SHEEP

5.2.1 PHYSICAL INSPECTION OF MILK

APPEARANCE OF NORMAL MILK

Normal milk should be white in colour, but may range to a white-yellow. Milk should have a thin consistency, with no solid milk clots whatsoever.

APPEARANCE OF COLOSTRUM

Colostrum has a thicker consistency than regular lactation milk, and is generally yellow in colour. Colostrum in sheep may be very thick and yellow – caramel-like, more so than in cattle or goats. If no clots are present this is not abnormal. This appearance only lasts a few days after lambing, then the milk returns to its normal colour and consistency.

Although colostrum may be considered 'normal' from a physiological aspect, it is not considered normal in terms of marketing (for human consumption). Ontario regulations consider colostrum to be 'abnormal' and therefore require that it not be mixed with milk in the bulk tank (milk for market). For cattle and goats the Ontario Provincial Milk Act describes abnormal milk as that which

a) comes from an animal 15 days prior to and 3 days after parturition, (or longer if it still contains colostrum)
b) contains blood or other foreign particles;
c) is watery or coagulated;
d) has odours that adversely affect its organoleptic characteristics;
e) is contaminated by chemical, toxin, drug or any other foreign substance.

Fig. 5. Secretion with gangrenous mastitis (N. East)

APPEARANCE OF ABNORMAL MILK

The characterization of abnormal milk can be quite subtle, from faint flakes in otherwise normal milk, to paste-like clots with no liquid present. The colour can range from white to white-yellow. In some cases, if there is an acute infection such as *E. coli*, milk is of thin consistency with little-to-no clots, but the liquid is yellow and clear. With severe gangrenous mastitis, the milk may look like red serum – with or without clots of milk (Fig. 25).

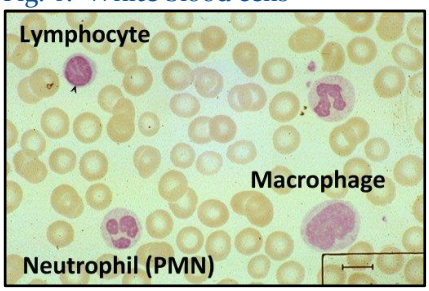
Sometimes, there may be a variation in the milk with the addition of fresh-appearing blood. This is due to trauma of the udder, however is not by infection, but from injury. This is quite common in early lactation animals after a difficult lambing, but can be caused by any injury to the udder.

5.3 DETECTION OF INFLAMMATORY SOMATIC CELLS IN AN INDIVIDUAL SHEEP

5.3.1 WHAT ARE SOMATIC CELLS?

Somatic cells, mostly comprised of white blood cells are defense cells of the immune system that are excreted into milk to kill bacterial infections in the udder. They also include cells sloughed from the alveoli (Section I.1.1). Somatic cells are always found in milk in low numbers; however, the number and type of cells varies greatly when there is infection in the udder. The following are cells that are found in normal milk:

Fig. 6. White blood cells



- Macrophages (big eaters in Latin); Approximately 80% of all cells in normal milk
- Lymphocytes; Approximately 15% of all cells in normal milk
- Polymorphonuclear leucocytes (PMNs) (also called neutrophils); <5% of all cells in normal milk
- Epithielial duct cells; <5% of all cells in normal milk

The primary role of macrophages and lymphocytes are to act as an alarm system in the udder, and signal when there are bacteria present in that gland. When this occurs, there is a huge influx of PMNs from the blood stream, and these cells destroy the bacteria in the tissue and the milk alveoli in the gland (see Section I for normal anatomy). At this time, the concentration of cells in the milk changes, and PMNs are the dominant cells found in the milk, until the infection subsides.

5.3.2 SOMATIC CELL COUNT (SCC)

Somatic cell count (SCC) is a diagnostic measurement to determine the approximate level infection in the udder. As previously mentioned, there are always somatic cells present in the milk, so there is no time that SCC will ever be zero. If SCC of a sample of the gland is below a particular threshold, it is assuming that the gland is healthy.

When an infection occurs, the influx of PMNs into the gland will trigger an increase in SCC, providing a good indication to producers that there is an infection in the gland. This is especially important for subclinical cases of mastitis, where there are no visible changes in the milk to indicate an infection, but the increase in SCC can make producers aware of a possible infection in the gland.

SCCs are measured in the amount of somatic cells that are

Table II.3. Relationship between linear score and somatic cell counts

LINEAR SCORE	SOMATIC CELL COUNT (CELLS/ML)	
0	12,500	Healthy Udder
1	25,000	
2	50,000	
3	100,000	
4	200,000	
5	400,000	Mastitis
6	800,000	
7	1,600,000	
8	3,200,000	
9	6,400,000	

found in one mL of milk. The unit of measurement is cells/mL of milk. A simplified way to understand these SCC is a linear score, which is a mathematical conversion of SCC. This value is easy to interpret both on an individual ewe level and flock level, and can be easily correlated with milk loss. Table II.3 is a list of linear scores, and its corresponding SCC value. Each doubling of the SCC results in an increase in linear score of 1. It also includes ranges for values for which it is more likely the udder is healthy and values for which it is more likely mastitis is present.

5.3.3 HOW ARE SOMATIC CELLS MEASURED?

SCC values can be determined at the individual gland, the individual ewe, and whole flock level. Milk should be aseptically taken into milk vials. These samples don't require refrigeration for transportation; however, it is important that they are preserved properly. Bronopol tablets are used to preserve the milk. Vials with these tablets can be purchased from a number of sources on-line or usually obtained from a laboratory that offers SCC counting. These laboratories will have automated cell counters, which detect particles in the milk and are accurate for sheep's milk. In Ontario, CanWest DHI[1] and the Agriculture and Food Laboratory, University of Guelph[2] offers this service.

Some sheep dairies may wish to invest in on-farm cell counting techniques. There are portable cell counters that can be easily used before milking to determine SCC status of a gland. A milk sample is taken aseptically, and then drawn up immediately into a plastic cassette, which is then inserted into the cell counter. A SCC value is then determined based on the milk in the cassette. This information can also be transferred to a computer for easy record keeping. A common cell counter that is available on the market is the DeLaval Cell Counter DCC.

5.3.4 WHAT IS A NORMAL SCC FOR DAIRY SHEEP?

When measuring SCC for udder health, it is important to have a benchmark SCC level, to know if a gland is "normal", or healthy. Any SCC over this benchmark can be an indication that there is an issue in the gland. Even though these benchmarks are a good guide, producers should strive for values under these levels for optimal udder health.

In dairy cattle, a normal udder health value to strive for is 200,000 cells/mL. In goats, the benchmark SCC level is significantly higher at 600,000 – 800,000 cells/mL. This benchmark increase is not surprising, as goats have a different secretory system than cows (apocrine vs. merocrine), which yields a higher SCC value, as described in Section I.1.1.

As sheep have the same secretory system as goats, it could be assumed that the benchmark SCC levels would be similar. However, research has shown that normal SCC values of sheep and goats are quite different. There is no established benchmark for sheep, however based on research – it is likely more similar to dairy cattle than to dairy goats. A flock value of < 500,000 cell/mL should be attainable, even when ewes are in late lactation. **Individual sheep values of > 400,000 (linear score ≥5) should be strongly suspected of having mastitis. Sheep can maintain an udder health SCC level < 200,000 cells/mL (linear score ≤4)** (See Table II.3).

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[1] http://www.canwestdhi.com/ Tel: 1-800-549-4373

[2] http://www.guelphlabservices.com/AFL/raw.aspx Tel: (519) 767-6299; Toll Free: 1-877-UofG-AFL (1-877-863-4235); Fax: (519) 767-6240; E-mail: aflinfo@uoguelph.ca

5.3.5 WHAT LEVELS ARE EXPECTED WHEN MASTITIS IS PRESENT?

An SCC values over the benchmark of 200,000 cells/mL is suggestive of a subclinical infection, although healthy udders may have values in this range. If accompanied by clinical signs of infection, it can be determined as a clinical infection. With subclinical mastitis, values > 400,000 cells/mL are often seen. Clinical mastitis often has SCC values > 1,000,000 cells/mL. However, interpretation of SCC values should also consider other influences as detailed below.

5.3.6 OTHER FACTORS THAT INFLUENCE SCC LEVEL

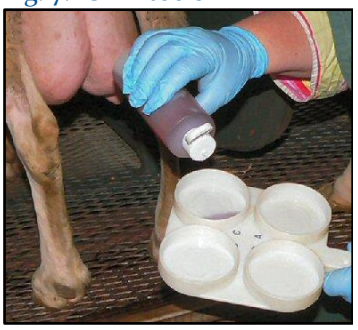
There are a variety of factors that can affect SCC levels in ewes other than mastitis.

- Stage of lactation: increased levels are generally found in both early and late lactation.
- Age of ewes: older ewes have had longer opportunity to be exposed to udder infections from previous lactations; they generally have a higher SCC than younger animals.
- Some breeds tend to have higher levels than others.

However, most important are the factors which also predispose to mastitis as has been covered in Section II.4.

5.3.7 CALIFORNIA MASTITIS TEST (CMT)

Fig. 7. CMT tools



A CMT is a practical tool that is used on-farm to detect ewes that have increased SCC. This method of monitoring udder health is simple, and producers can get almost instantaneous results, and allows them to pinpoint their high SCC animals, and those with subclinical infections in a very easy manner.

HOW DOES A CMT WORK?

A CMT is a system that uses a paddle split into four wells to test individual glands for SCC level in an animal (Fig. 27). Initially designed for dairy cattle, it can be easily used with sheep, by just using two of the wells. Milk is mixed with a CMT reagent (purple solution). If somatic cells are present a thickening or gelling of the milk will occur. The CMT reagent reacts with the nucleic acid of the somatic cells present in the milk. The higher the SCC, the more gelling that occurs. These reactions are categorized into five categories, from negative to a strong positive test. (Table II.4).

The CMT reagent also indicates how acidic or alkaline (pH) the milk is. Normal milk is slightly acidic (pH 6.6 to 6.8). As SCC climbs, the milk becomes more alkaline and the reaction appears more intensely purple.

One of the main benefits of the CMT is that the reagents will not react with other substances such as blood or manure. Having said that, it is important to make sure that the paddle is as clean as, as excess debris could affect how to milk and reagent solution moves within the quadrants of the paddle.

HOW TO PERFORM A CMT

Fig. 8. Strip cup to inspect milk

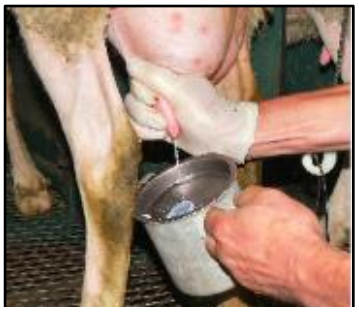
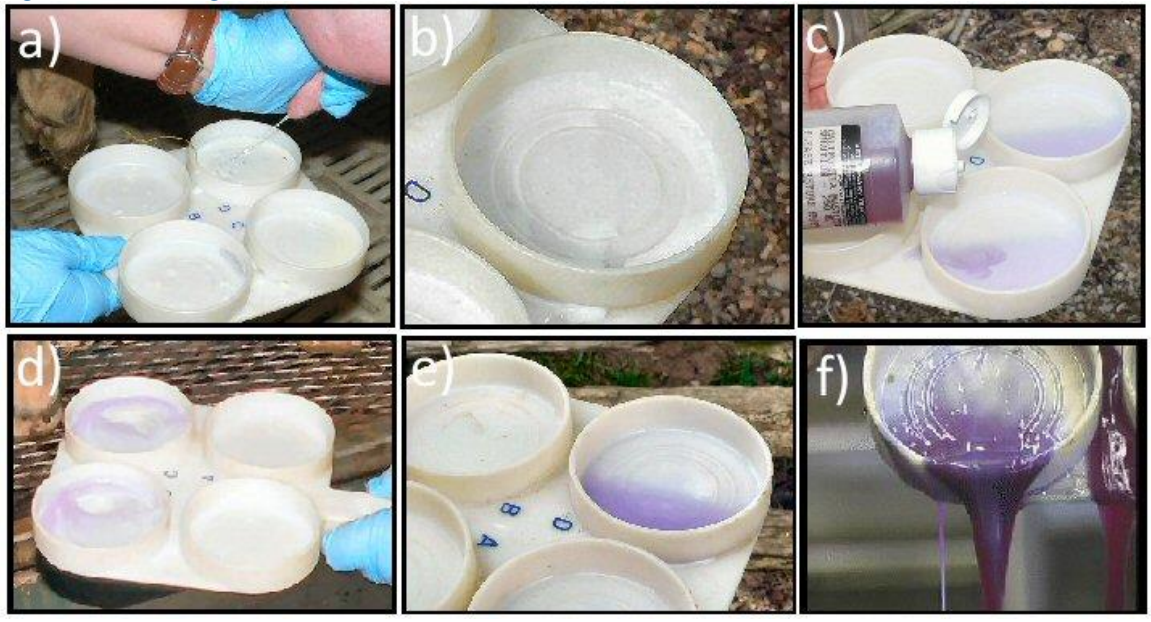


Fig. 9. Clinical mastitis

- Gloves should be worn at all times
- The udder and teats should be clear of dirt before sampling
- The foremilk should be removed prior to testing (Fig. 28).
- Use a strip cup to allow visualization of the milk against a black background. If the milk appears abnormal with clots and discolouration, mastitis is present and the CMT is not necessary to perform (Fig. 29).
- Place the paddle underneath the ewe, and sample milk into one well per gland (Fig. 30a).
- The paddle can be held in any direction, but be consistent so it is clear which gland corresponds to each well.
- At least 5 mL (1 teaspoon) should be expressed into each well.
- Pour out excess milk by tilting the paddle, ensuring that there is enough milk covering the complete area of the quadrant (Fig. 30b).
- Keeping the paddle tipped, add an equal amount of reagent to the milk (i.e. 5 mL which is about a teaspoon), making sure that the total amount of liquid does fill more than half of the well (Fig. 30c).
- Bringing the paddle back to a level position, mix the milk and reagent together by moving the paddle gently in a circular motion, for approximately 10 – 30 sec (Fig. 30d)
- Observations of the consistency of milk can be done while swirling, to see if any gel is forming. Alternatively, the paddle can be tilted to observe the consistency of the liquid as it is pouring out

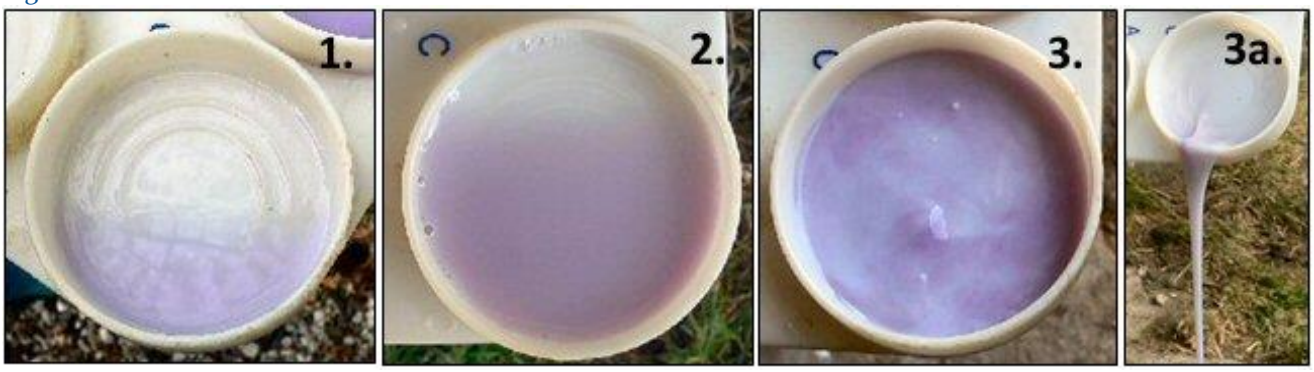
Fig. 10. Performing a California Mastitis Test (CMT)



USING THE RESULTS OF A CMT

The CMT can be used for daily monitoring of udder health on-farm (Fig. 31). If you suspect that a ewe may have an udder infection, the milk can be tested immediately. If the reaction is ≥ CMT 1+, a milk sample should be cultured. CMT is a good screening process to ensure that only glands that are high in SCC levels are cultured, which decreases the cost.

Fig. 11. CMT reaction



However, it is important to understand that this technique does have its limitations. Interpretation of the score is subjective. False positives can occur in ewes that are early or late in lactation. SCC is a more reliable test than CMT. Table II.4 is adapted from the Canadian Bovine Mastitis Research Network TACTIC Udder Health Veterinary Kit, and describes the visual identification of the range of CMT scores.

Table II.4. California Mastitis Test scoring system

SCORE	INTERPRETATION	VISUAL CHARACTERISTICS OF LIQUID	SCC RANGE (CELLS/ML)
N	Negative Sample (Fig. 31 #1)	The mixture does not change, and remains the same liquid consistency of milk with bluish/purple tinges.	0 – 200,000
T	Trace Sample	The mixture will thicken slightly like very thin porridge, however, it can revert back to its original state when moving the paddle.	150,000 – 500,000
1	Weak, but Positive Sample (Fig. 31 #2)	There is slight thickening of the milk like thin porridge; no gel forms; when swirled, the mixture will climb the walls of the well; and when poured out, the mixture flows a steady pace.	400,000 – 1,500,000
2	Distinctly Positive Sample	Gel is beginning to form; when swirled the gel tends to clump in the middle of the well; when poured out, the gel will pour out first, leaving some liquid is remaining in the well.	800,000 – 5,000,000
3	Strongly Positive Sample (Fig. 31 #3 and #3a)	The entire mixture is gel; when swirled it clumps in the middle; and when poured out of the paddle, no liquid remains in the well.	> 5,000,000

5.4 DETECTION OF UDDER PATHOGENS IN THE INDIVIDUAL SHEEP

Commonly, detection of pathogens in the udder involves culturing of the milk for presence of bacteria and mycoplasma. It is unusual that we choose to detect viruses from milk although tests have been

developed for detection of maedi visna virus using a polymerase chain reaction (PCR), used to detect the DNA of the virus.

5.4.1 OBTAINING AN ASEPTIC MILK SAMPLE FROM AN INDIVIDUAL EWE FOR CULTURE

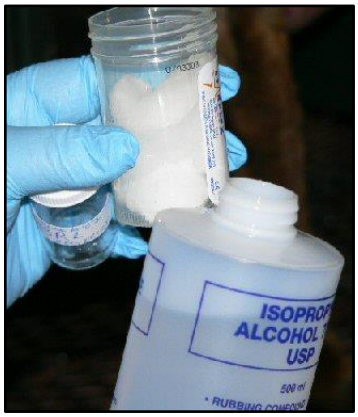
When taking a milk sample for submission for culture, it is important that preparation of the teats is done correctly to ensure that samples do not become contaminated. Ideally, these samples would be taken at the time of milking, as udder preparation and disinfection are being done at that time and milk let-down is maximized, which helps with manual teat sampling.

MATERIALS REQUIRED

The following materials are required for aseptic milk sampling on sheep flocks:

- Sterile milk vials; ideally a snap-cap vial, which is easier to open, as compared to a twist top vial (Fig. 32)
- Gloves
- Labels and markers for labelling the vials
- Udder wash solution and cloths/towels to initially disinfect and dry the udder and teat
- Sterile swabs that are soaked with 70% isopropyl alcohol to disinfect the teat
- Cooler with ice packs to put milk samples in when transporting from the farm

Fig. 12. Materials for milk culture

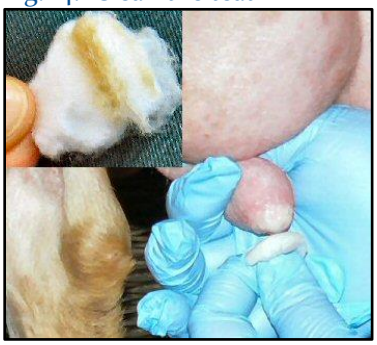


PREPARING THE UDDER AND TEAT

- Before doing any type of udder and teat preparation, it is important to remove any excess dirt or manure from the udder, as it could fall into the milk vial during milking, and contaminate the sample.
- Gloves should be worn at all times during sampling
- The udder and teats should be clean and dry, using udder wash or wipes and a single service cloth or towel (Fig. 33).
- Four or five strips of milk should be extracted from the teat before taking the sample, as the milk closest to the teat end has a chance of having increased pathogen loads.
- Teats should be fully disinfected with sterile swabs.
- Teat ends should be thoroughly wiped with a new sterile swab, which should kill the majority of pathogens on the exterior of the teat. Use new swabs until the swab is clean (Fig. 34).

Fig. 13. Clean the udder

Fig. 14. Clean the teat



TAKING THE SAMPLE

- All sampling should be done in the same fashion as stripping foremilk during udder preparation or hand milking, by manually stripping the teat in a downward motion (Fig. 35).

- When removing the cap of the milk vial, it is essential that the lid is held with the inside of the cap downwards, to prevent any debris from getting inside.
- The vial itself should be held at a horizontal angle to ensure that no debris will get into the vial.
- Ensuring that the teat end does not come in contact with the tube, begin stripping the milk into the tube.
- Only fill the milk vial up to approximately ¾ of the full volume, as there is a chance of the vials to open when frozen if they are too full.
- If composite samples are being taken instead of individual gland samples, ensure that equal amounts of milk are taken from each gland.
- Teats should then be disinfected after sampling with post-milking teat dip.

Fig. 15. Take a sterile milk sample

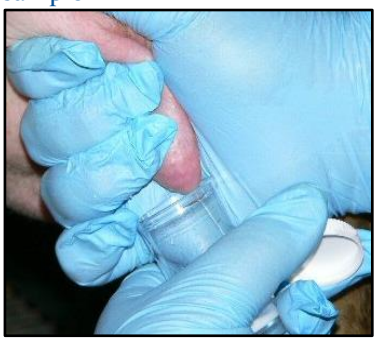


Fig. 16. Labelled for submission

HANDLING OF SAMPLE INCLUDING STORAGE AND SHIPPING TO LABORATORY

- It is important to properly label the milk samples to make sure that each sample is identified properly (Fig. 36). The following information should be included on the label:
  - Date
  - Farm name
  - Ewe unique identification number
  - Gland sampled (left or right)
  - Reason for sampling (i.e. mastitis case, high SCC, positive CMT reaction)
- Samples should be placed in a cooler with frozen ice packs, and sent to a lab as soon as possible. If samples are being held at the farm for an extended period of time, they should be frozen immediately.
- For submitting samples, it is important the flock veterinarian fill out the appropriate paperwork for laboratory submission.
- If milk samples are stored in a 4 °C fridge, they should remain there no longer than 24 h before culturing.
- If the sample is frozen in a -20 °C freezer (normal temperature of a home freezer), it should remain there no longer than 1 month before submitting for culture.

Fig. 17. Culturing milk on blood agar plate at the laboratory

- If testing for mycoplasma rather than bacterial pathogens, it is essential that the milk samples remain unfrozen but they must be processed by the laboratory within 72 h of taking the sample.

5.4.2 HOW TO INTERPRET MILK CULTURE RESULTS

Results from the lab give information of what bacteria are found in each sample, and the number of each type of bacteria that are found on the milk culture. Generally we can distinguish culture results into three categories:

- Culture positive

- No growth
- Contaminated

CULTURE POSITIVE RESULT

One bacterial type (e.g. colony type) is prevalent on the plate, and is assumed to be the cause of the mastitis infection. If the plate has two different types of bacteria present, one being an important pathogen, it is considered the source for the mastitis infection.

Fig. 18. Positive culture results

Source: http://www.healthhype.com/

NO GROWTH RESULT

Even with samples from cases of clinical mastitis, there are times that the samples will show no growth on the plates, despite there being an obvious infection. There are a variety of reasons for this result, including:

- Inadequate volume of milk submitted for culture
- Non-bacterial infections, such as viruses (e.g. maedi visna), yeast or mycoplasma
- Acute and systemic infections that have already been cleared by the body at the time of milk sampling, such as *E. coli* infections. The SCC levels may still be very high but the bacterial infection has been cured
- The ewe has been recently treated with antibiotics and their presence in the milk is stopping the growth of bacteria on the plate
- Not drying the teats well enough and contaminating the milk with a disinfectant

CONTAMINATED RESULT

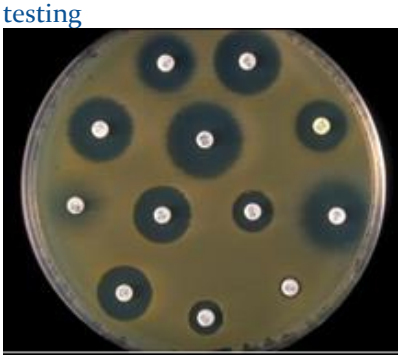
Contaminated samples are generally defined as three or more bacterial species or colony types on a culture plate from a milk sample. There are usually three or more different types of bacteria. Some of these bacteria could be found in the milk, but some could be from debris that fell into the vial while taking the sample, dirt still remaining on the teat end or from dirty hands recontaminating the teat and/or lid of the vial.

NUMBER OF BACTERIA / COLONY FORMING UNITS

The number of **colony forming units** per millilitre of milk (CFU/mL) that are reported by the laboratory is also important when interpreting results. In Fig. 38, each "dot" on the plate is one CFU. The CFU/mL is reported as the number of bacterial colonies that grow on culture plates, multiplied by 100 to account for volume of the sample that was actually plated for culture. For example, if there are two colonies on a culture plate, this result is reported as 200 CFU/mL. In general, if there are 1,000 CFU/mL or greater (i.e. > 10 bacterial colonies on the plate) for a single bacterial type, it can be assumed that that gland is infected with that bacteria, however, some research suggests that a CFU cut-point can be anything over 200 CFU/mL.

5.4.3 ANTIBIOTIC SENSITIVITY TESTING

Fig. 19. Antibiotic sensitivity testing



Source: http://textbookofbacteriology.net/index.html

Antimicrobial sensitivity testing is a procedure done to determine whether or not mastitis-causing bacteria are sensitive or resistant to a specific antibiotic (e.g. penicillin, tetracycline, etc.).

To do this test, a broth mixture containing the bacteria previously isolated from the milk sample, is poured over an agar plate. Small paper disks the size of a pencil end that have specific antibiotics added to them are placed on the plate. The antibiotic leaches out into the agar around the disk. Bacteria will grow in the agar where there are no antibiotics to retard their growth but not grow around the disks where the antibiotic is present – unless that bacteria are resistant to that antibiotic in which case they will grow right up to the disk. So a clear zone around the disk is good news – that antibiotic may be effective. No zone around the disk is bad news, that bacterial isolate is resistant to the antibiotic. The lab report will come back that the bacteria is either sensitive (S), resistant (R), or is moderate sensitive (I) to that antibiotic depending on how close they grow to the disk containing the antibiotic. This information can help when selecting the correct treatment for that case of mastitis.

5.4.4 ROUTINE FLOCK CULTURE

WHOLE FLOCK CULTURE

Routine culturing of lactating ewes will improve understanding udder health in a flock. Culturing milk samples from all lactating ewes at once (whole flock culture) may be recommended for flocks in which SCC levels at the flock level (e.g. bulk tank) are markedly elevated or when there is a high incidence of clinical mastitis. In this case, culturing the entire lactating flock can identify problem animals. Whole flock cultures are done initially on composite samples (both glands in the same vial), and will identify ewes that may need to be treated, culled or identified and milked last.

TARGETED CULTURE OF INDIVIDUAL EWES

Certain animals or certain times of lactation may be targeted for culturing as part of a program to screen for mastitis cases. This routine sampling can be done when whole flock culturing is not required. Some key screening criteria to culture animals are as follows:

- Ewes with clinical mastitis
- Ewes with abnormally high SCC's (e.g. ewes > 400,000 cells/mL or ewes > 200,000 cells/mL if mastitis levels are low (Section VII))
- Ewes with CMT reactions 1+ or higher
- Ewes at lambing or at dry-off
- Purchased ewes