

Faculty of Veterinary Medicine,
Szent Istvan University,
Department of Animal Hygiene.

An Evaluation of traditional, novel and prospective cow-side tests in
an approach to mastitis diagnosis.

Danielle Dillon

Supervisor: Peter Kovacs, Department of Animal Hygiene, Budapest.

2012.

Table of Contents

1. Introduction	3
2. Mastitis	5
2.1 Mastitis Definition.....	5
2.2 Pathogenesis.....	6
2.3 Major mastitis pathogens.....	8
3. Mastitis induced changes in milk	11
3.1 Overview.....	11
3.2 Physical changes in milk.....	12
3.3 Changes in Somatic cell count (SCC).....	13
3.4 Conduction changes in milk.....	15
4. Milk sampling technique	16
5. Clinical examination	17
6. Methods of mastitis diagnosis using Somatic cell count (SCC)	19
6.1 Conventional methods.....	19
<i>California Mastitis Test (CMT)</i>	19
Theory.....	19
Method.....	19
Experimental findings.....	21
Summary.....	22
<i>N-acetyl-Beta-D-glucosaminidase (NAG-ase)</i>	23
Overview.....	23
6.2 Novel cow-side tests.....	25
<i>PortaSCC®</i>	25
Theory.....	25
Method.....	25
Experimental findings.....	27
Feedback.....	29
Summary.....	31
<i>Delaval cell counter</i>	32
Overview.....	32
6.3 Future Prospects.....	33
1) <i>Lactate dehydrogenase (LDH) measurements as an indication of mastitis</i>	33
2) <i>Frunzzyhans improved bioluminescent assay</i>	35
7. Cow-side methods of mastitis diagnosis using Electrical conductivity (EC)	36
1) Milking unit.....	36
2) The milking robot.....	37
3) Experimental findings.....	38
8. Discussion	39
9. Conclusion	42
10. Summary	44
11. Acknowledgements	45
12. References	46

1. Introduction

Bovine mastitis is a significant disease of dairy herds, having a large adverse effect on farm economics, due to a reduction in milk production and treatment costs (Viguier, 2009). There are a large number of methods of detection currently in use, in order to monitor udder health performance.

Changes in milk composition as a consequence of mastitis have contributed greatly to the diagnosing process. Physical changes detected in the milk, in which it is clumpy, foul smelling and salty tasting are all findings consistent with mastitis (Blowey, 2010).

Conventional methods of mastitis detection include estimation of somatic cell count (SCC), bacterial culture and measurement of biomarkers associated with the onset of disease e.g., N-acetyl-B-D-glucosaminidase (NAG-ase) and lactate dehydrogenase (LDH). However several of the conventional methods of mastitis detection have their limitations (Viguier, 2009).

Current tests involving SCC as an indication of mastitis involve direct methods, which are extremely accurate, however they are expensive (e.g., Fossomatic cell counter), and are not always available cow-side (Lam, 2009).

Indirect methods such as the California Mastitis Test (CMT) are effectively used for SCC determination also. This assay indirectly measures the SCC in milk samples.

A bromocresol-purple-containing detergent is used to break down the cell membrane of somatic cells. The subsequent release and aggregation of nucleic acid forms a gel like matrix, with a viscosity that is proportional to the leukocyte number. This test is cost effective (approx. \$12 per 350 tests), user friendly, and easy onsite application (Viguier, 2009).

However results can be difficult to interpret, the method itself has a low sensitivity (Viguier, 2009), and it cannot be used to detect pathogens in milk from cows earlier than four days post calving (Lam, 2009).

Novel cow-side tests such as PortaSCC® and the Delaval Cell Counter are accurate on farm indicators of mastitis (Lam, 2009).

Recent developments in the field of early detection of mastitis using SCC have been brought to attention. A recently developed bioluminescence assay by Frundzyhan involving SCC has proven to be extremely accurate in its diagnosis and its onsite potential is promising. Another major contribution to this field, involves an experiment conducted by Hiss in which he

identifies sub-clinical mastitis with the use of a portable spectrophotometer in measuring LDH activity.

Measurement of electrical conductivity (EC) is currently used in mastitis diagnostics (Lam, 2009). The principle is based on the measurement of increased conductivity in milk due to the elevation of certain ions that result from the inflammatory process (Sandholm, 1995).

The main advantage of this method is its ability to be used 'on-site' (Viguier, 2009).

All cow side tests aim to be of high sensitivity, easy to use, and to have onsite practical application.

2. Mastitis

2.1 Mastitis Definition

Mastitis (*mast* = breast; *itis* = inflammation): inflammation of the mammary gland.

Bovine mastitis is almost always due to the effects of infection by bacteria (Viguier, 2009). Epithelial cells lining the alveoli within the udder undergo pathologic changes that bring about a decrease in functional capacity.

The majority of infections result in a mild (or sub-clinical) inflammation. However, functional losses may continue into further lactations, depending upon the pathogen involved, which in turn may reduce productivity and weight gain. In the most extreme cases it may lead to agalactia, or a serious systemic involvement, resulting in death (Merck, 2011).

Occurrence of mastitis seems to be influenced by the following factors: climatic conditions, seasonal variation, bedding, housing, livestock density and husbandry practices (Merck, 2011).

According to the severity of inflammation it may be divided into sub-clinical and clinical forms.

Sub-clinical mastitis is difficult to detect due to the absence of visible indications. Chronic mastitis is a rare form of the disease and results in persistent inflammation of the mammary gland (Viguier, 2009).

With **clinical** mastitis, the balance between host defences and invading pathogens attracts a marked inflammatory response and clinical signs become apparent (Merck, 2011). Clinical cases result in pain, heat and swelling of the affected udder and abnormality of milk with either clots or flakes (Sandholm, 1995).

Clinical mastitis, in which abnormal milk is readily detected, and sub-clinical mastitis, in which no change in the milk is apparent, both reduce milk production (Viguier, 2009).

Clinical cases can be easily identified by the naked eye (Lam, 2009), however sub-clinical cases are difficult to detect due to the absence of visible signs (Viguier, 2009). For this reason there is an emphasis placed upon “appropriate detection” for sub-clinical cases (Hiss, 2007).

Healthy udders are economically profitable and lead to a better quality product (Lam, 2009). For these reasons accurate early diagnosis is imperative.

2.2 Pathogenesis

Normally sphincter muscles tightly close the teat canal to prevent the entry of micro-organisms. The teat canal is lined with keratin that obstructs the migration of bacteria. During milking this keratin plug is flushed out and there is distension of the teat canal. The teat canal remains open after milking for approximately two hours (Sand Holm, 1995).

As parturition draws near, fluid accumulates within the mammary gland resulting in an increased intra-mammary pressure. The mammary glands defences are weakened due to dilation of the teat canal and leakage of mammary secretions (Viguier, 2009).

In the case of bacterial infection, once inside the teat they begin to multiply. One can see from **Figure 1**, the schematic representation of mastitis development in an infected udder.

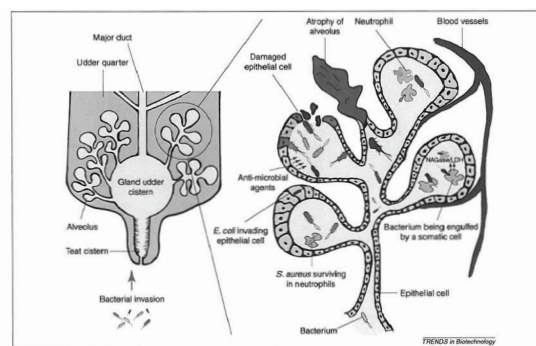


Figure 1. Schematic representation of mastitis development in an infected udder. Environmental and contagious microorganisms invade the udder through the teat cistern. They then multiply in the udder where they are attacked by neutrophils while damaging the epithelial cells lining the alveoli, with subsequent release of enzymes like MDAase and LDH. The epithelial cells also secrete anti-microbial compounds. Considerable tissue damage is observed once the immune effector cells begin to combat the invading pathogens.

Figure 1. Mastitis development.

(Viguier, 2009)

The bacteria release toxins and induce leukocytes and epithelial cells to release chemo-attractants, including cytokines such as Tumour necrosis factor alpha (TNF alpha), Interleukin -8 and -1, eicosanoids like PGF2 alpha, oxygen radicals and acute phase proteins like haptoglobin and serum amyloid A. This attracts immune effector cells, mainly polymorphonuclear neutrophils (PMNs) to the site of infection (Viguier, 2009).

Polymorphonuclear neutrophils engulf and destroy the invading bacteria. They contain intracellular granules that store bactericidal peptides proteins, enzymes (myeloperoxidase) and proteases (elastase, cathepsin G, B and D) (Viguier, 2009). These compounds when released destroy bacteria and some epithelial cells, resulting in decreased milk production and release of enzymes such as LDH and NAG-ase. PMNs die by apoptosis and engulfment by macrophages once their task is completed (Viguier, 2009).

The reaction of neutrophils is considered to be the most important defence and cleaning mechanism of the udder (Sandholm, 1995). The dead mammary epithelial cells in addition to the dead leukocytes are secreted into milk resulting in a high SCC (Viguier, 2009). However the majority of cells resulting from a high SCC are usually those of neutrophils that have moved from the blood to the milk (Sandholm, 1995).

2.3 Major mastitis pathogens

The most frequent bacterial causes for mastitis have been segregated into two specific groups: contagious and environmental mastitis pathogens (Faull, 1991).

The result of infection can be a sub-clinical case where the milk is positively infected with pathogens, has increased inflammatory and somatic cell count, but looks normal and the quarter feels normal (Faull, 1991).

Clinical mastitis can be visually detected according to clots and flakes present in the milk (Faull, 1991).

The most prevalent **contagious** mastitis organisms are as follows: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Coagulase negative staphylococci* and *Streptococcus dysgalactiae*.

Other less common contagious pathogens include: *Corynebacterium bovis* and *Mycoplasma* species (Blowey, 2010).

The contagious pathogens live and multiply on or in the udder and teats. They are spread at milking usually via teat cups or milkers hands (Faull, 1991).

Most cases caused by contagious mastitis pathogens are sub-clinical. “ Herds that only have a problem with contagious infections typically have a high cell count but often have a low incidence of Clinical mastitis” (Blowey, 2010).

The control of contagious pathogens involves the regular use of post milking teat dip and dry cow therapy (Faull, 1991) as well as milking hygiene and culling procedures (Blowey, 2010)

1a) *Staphylococcus aureus* (Blowey, 2010).

- *Staphylococcus aureus* are haemolytic, gram-positive cocci.
- Under certain conditions where the immune status of the cow is compromised, *Staphylococcus aureus* infection can proceed to an acute gangrenous mastitis.
- Poor response to antibiotic treatment has been observed with this pathogen. In a chronic infection, fibrous tissue can create a ‘wall’ that prevents the antibiotic from penetrating the bacteria. In addition to this *Staphylococcus aureus* can inhabit PMNs, macrophages and epithelial cells that are out of reach from certain antibiotics.

1b) *Coagulase-negative Staphylococci* (*S. xylosus*, *S. intermedius*, *S. hyicus* and *S. epidermis*) (Blowey, 2010).

- They commonly colonize the teat skin, teat end and teat canal.
- They can cause both clinical and sub-clinical mastitis.
- These pathogens are known to be present in maiden and pregnant heifers, however intra mammary treatment of a pregnant heifer can cause problems such as removal of the natural keratin plug and exposure of the teat canal for new infection.

1c) *Streptococcus agalactiae* (Blowey, 2010).

- Gram-positive alpha-haemolytic coccus.
- This is a highly contagious pathogen, which mainly resides in the udder.
- High incidences of sub-clinical infection have been reported with this pathogen.
- Appropriate hygiene at milking is an important control point. “If *S. agalactiae* is isolated from a herd, it is a good indication that there has been a breakdown in the basic hygiene of the milking routine” (Blowey, 2010).
- Good response to antibiotic treatment.

1d) *Streptococcus dysgalactiae* (Blowey, 2010).

- Gram-positive haemolytic coccus.
- This type of mastitis infection is usually associated with teat lesions.
- This pathogen survives exceptionally well in the environment.
- *S. dysgalactiae* can be found as a component of summer mastitis complex.

The most prevalent **environmental** mastitis pathogens are: *Streptococcus uberis* and *Escherichia coli*.

Others include: *Citrobacter*, *Enterobacter*, *Klebsiella*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus licheniformis*, *Pasteurella*, *Streptococcus faecalis*, *Fungi* and *Yeasts* (Blowey, 2010).

These environmental pathogens live and multiply in the faeces, bedding, water supply, flies and any other suitable environmental source (Faull, 1991). Infection is inflicted upon those cows that have been contaminated by the infected environmental source (Faull, 1991).

Transfer of infection usually occurs between milkings and during the dry period (Blowey, 2010). A high proportion of cases are clinical, however *Streptococcus uberis* can cause sub-clinical cases (Blowey, 2010).

Environmental mastitis is controlled via general hygiene, ventilation, and dryness, fly control (Faull, 1991). In addition to this a most important method of control involves application pre and post milking dip and dry period teat seal, as many new infections are introduced in the dry period (Blowey, 2010).

2a) *Escherichia coli* (Blowey, 2010).

- *E. coli* is an environmental organism found from faeces, thus is strongly associated with cows housed in humid and soiled conditions.
- Symptoms of *E. coli* mastitis include a hard, hot and swollen quarter with watery discharge. In some cases cows show severe signs of shock and succumb rapidly.
- The toxic effects of *E. coli* are due to the release of endotoxins. Lipopolysaccharide endotoxin is released from the bacteria cell wall and phagocytized by PMNs, which consequently release lysosomal granules that enhance the shock reaction.
- A vaccination has been developed against *E. coli* infection. A reduction in clinical and acute toxic cases can be achieved with the use of this vaccine.

2b) *Streptococcus uberis* (Blowey, 2010).

- The most common environmental pathogen causing mastitis, and the most common cause of infection of dry cows is *Streptococcus uberis*.
- A typical case presents itself with a sudden onset with a high body temperature, where the quarters are hard and swollen, and the milk is laden with large clots.
- This infection has been linked to animals housed on straw bedding. A method of control is to apply some lime or ash to keep the pH of the bedding high and thus prevent bacterial growth.

Yeasts (*Candida* species) and moulds (*Aspergillus fumigatus*) and *Prototheca zopfii* (algae) are common environmental causes of mastitis. This mastitis is typically seen as a hard, hot and swollen quarter, with thick white clots. The cow may have an elevated body temperature. An 'off label' treatment known to be effective is a mixture of 1.8 grams of iodine crystals dissolved in 2 litres of liquid paraffin & 23ml of Ether, to be flushed into the quarter and stripped out for two to three days (Blowey, 2010).

3. Mastitis induced changes in milk

3.1 Overview

“ Changes in milk include increased conductivity, increased pH, raised water content, and the presence of visible clots and flakes” (Viguier, 2009).

Physical changes, somatic cell count (SCC) alteration and increased electrical conductivity (EC) are all alterations consistent with the findings of mastitis.

Several processes are occurring simultaneously to bring about these changes.

The change in enzyme levels and biochemical activity bring about the physical changes that one can detect by the naked eye, odour, touch or taste (Sandholm, 1995).

Somatic cell count alteration is due to inefficient and dead epithelial cells and leukocytes that are secreted into the milk (Viguier, 2009).

Due to the increased permeability of the blood vessels: ions, proteins and inflammatory cells merge from the blood to the milk, leading to an increased SCC and EC of the milk (Sandholm, 2009).

Once the blood milk barrier has been breached, one can detect the physical changes in milk.

“ When extensive damage to the blood-milk barrier has occurred, blood might be detected in the milk” (Viguier, 2009).

3.2 Physical changes in milk

The decrease in **milk yield** may be one of the first signs observed by the farmer. With udder inflammation there is a decrease in milk production (Sandholm, 1995). This should be one of the first pieces of information gathered in a clinical examination to assist in the diagnosis of mastitis.

In the event of mastitis the major components of milk are altered as a consequence of diminished synthesis (Sandholm, 1995).

The lipid composition changes to lower the quality of milk products (Sandholm, 1995).

Milk lipid production takes place in the udders secretory cells. Fatty acids are combined with glycerol and converted into a triglyceride (Blowey, 2010).

A rancid **flavour** is detected as the increased lipase degrades lipids into fatty acid components (Blowey, 2010).

Most of the protein found in milk is in the form of casein (Blowey, 2010).

Typically the amount of casein diminishes due to an increase in the proteolytic activity (Sandholm, 1995). The increase of plasmin breaks down casein (Blowey, 2010). The fractions of albumin and globulins increase in the milk, as many of these proteins cross directly from the blood to the milk (Blowey, 2010).

The alteration in protein fractions may result in a constant total protein amount, however the milk is much poorer in quality than that of normal milk (Blowey, 2010).

The foul **odour** and structure of the infected milk, is a consequence of the increase of enzymatic activity (Sandholm,1995). There is a marked increase in: oxidizing (catalase, phosphatase, peroxidase and xanthine oxidase), lipolytic (lipoprotein lipase and bacterial lipase) and proteolytic enzymes (proteinase, plasmin and bacterial proteinase) (Sandholm, 1995).

A salty, bitter taste imparted in the milk is a likely sign of mastitis. Lactose is the most important osmotic component in milk (Sandholm, 1995). In the incidence of mastitis, the lactose concentration decreases in milk and consequently sodium and chloride ion levels increase in the milk in order to maintain an adequate osmotic pressure (Blowey, 2010), hence giving rise to a salty taste.

Water soluble vitamins mainly ascorbic acid and riboflavin are influenced by the mastitis process. They can decrease in milk by up to 50%. They contribute to the bacterial fermentation process, which in turn results in a sour milk product (Sandholm, 1995).

3.3 Changes in Somatic cell count (SCC)

A major factor attributed to increased somatic cell count (SCC) is that of mastitis (Blowey, 2010).

“The SCC in truly uninfected quarters does not exceed approximately 70’000 cells/ml.” (Lam, 2009).

Penetration of bacteria into the udder leads to a rapid rise in SCC.

“Dead and sloughed off mammary epithelial cells, in addition to the dead leukocytes are secreted into milk, resulting in high milk SCC” (Viguier, 2009).

It is thus understood that the prevalence of these dead epithelial cells and leukocytes in milk result from the udders defence mechanism to combat the infection (Blowey, 2010).

The inflammatory response takes place in three steps (Sandholm, 1995).

- 1) The endothelial reaction occurs at the affected site. There is an increase in capillary permeability as the endothelial cells contract. Plasma and protein leak through inter-endothelial gaps resulting in interstitial oedema. Leukocytes adhere to the endothelium.
- 2) Migration of phagocytic cells from the circulation to the infection site.
- 3) Sequential tissue degeneration, regeneration, and the formation of fibrotic tissue (characteristic to the chronic proliferative phase).

If the udder defence mechanism is able to overcome and eliminate the infection, the SCC will return to normal (Blowey, 2010).

However when the inflammation fails to eliminate the pathogen, sub-clinical mastitis will result (Blowey, 2010), in which case leukocytes are continuously being secreted into the milk, leading to an elevated SCC (Sandholm, 1995).

Different bacteria affect SCC respectively. Contagious bacteria are more likely to produce a sub-clinical infection than environmental bacteria with the exception of *Str. uberis* (Blowey, 2010).

Infections by environmental organisms tend to be rapidly eliminated, and usually raised cell counts are only observed in the mastitis period (Blowey, 2010).

In certain incidences there is a correlation between somatic cell count and level of infection in the udder. For e.g., *Strep. agalactiae* infection may produce up to 12’000’000 cell/ml in infected quarters and this correlates with the severity of the infection in the udder.

In other incidences, for e.g., in a *Staph. aureus* infection, the bacteria are shed in lower numbers and produce a variable response (Blowey, 2010).

Cow-side tests and prospective tests for determining SCC are being frequently used and developed. The CMT a more traditional method has been proven successful for over 40 years (Lam, 2009).

New current tests such as the PortaSCC® are becoming frequently more popular amongst farmers and practitioners today (Lam, 2009).

Other factors are known to have an impact on SCC. Lactation can affect SCC (Sandholm, 1995), from **Figure 2**, it is seen that a negative correlation is established between milk production and SCC of milk during lactation.

The cell reaction against infectious pathogens in cows that have had mastitis increase with age, however age doesn't affect SCC if a cow is healthy (Sandholm, 1995).

The time between milkings is a factor that affects the SCC. Usually the SCC remains high for three to four hours after milking (Sandholm, 1995).

It is known that some breeds have a lower SCC than others for e.g., Finnish Ayrshire cows are shown to have a lower SCC than Friesian cows as depicted below in **Figure 2** (Sandholm, 1995).

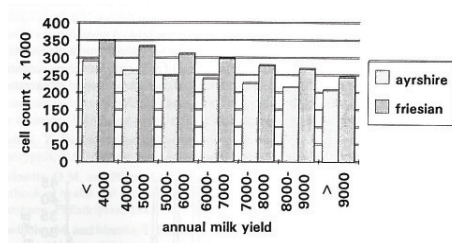


Figure 2. Milk production and cell count during lactation.

(Sandholm, 1995)

In addition to these factors, SCC can easily be affected by other conditions such as stress, nutrition and parity (Chagunda, 2005).

3.4 Conduction changes in milk

The sodium and chloride ion concentration increase in milk as a result of udder inflammation (Blowey, 2010). Potassium levels drop and the pH can increase from 6.6 – 7 in the course of a strong inflammation (Sandholm, 1995). The affected milk results in an increased level of conductivity. With mastitis the electrolyte content is higher and the fat content is lower than that of normal milk (Sandholm, 1995).

During the course of mastitis, the udders ability to concentrate ions is weakened and there is an increase in the passive permeability of blood vessels (Sandholm, 1995).

Lactose is the main osmotic determinant in milk (Sandholm, 1995). With its decrease, sodium and chloride ions cross from the blood to the milk in order to maintain the osmotic pressure (Blowey, 2010).

Calcium, phosphorus, magnesium and potassium levels are decreased. There is an increase in copper and iron, and a decrease in zink (Sandholm, 1995).

The pH increases in mastitic milk, due to the transfer of bicarbonate ions across the blood milk barrier (Sandholm, 1995). Thus, there is an increased quantity of ionising salts present in the milk, and this can be used to evaluate the mastitis status by electrical conductivity measurements (Blowey, 2010).

Figure 3 portrays those factors, which influence the osmolality of milk.

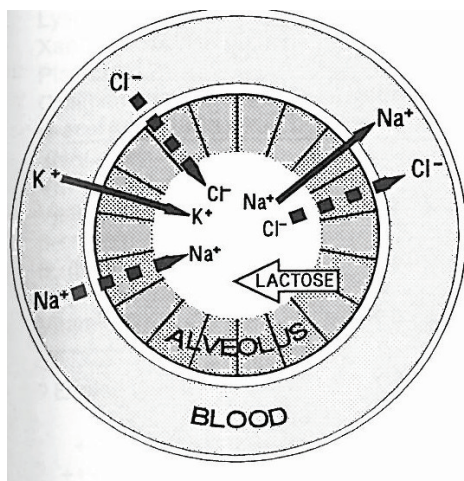


Figure 3. Schematic representation of ionic exchange across the blood milk barrier (Sandholm, 1995).

4. Milk sampling technique

Quarter, composite or bulk tank milk samples can all be obtained for mastitis diagnosis

Under practical circumstances most milk samples are obtained at quarter level (Sandholm, 1995).

“Milk samples for SCC measurement should be taken immediately before milking, after removing three squirts of milk” (Lam, 2009).

SCC is higher in the strippings and towards the end of milking.

Foremilk samples are preferred for sampling, after removal of the fore-strip (Sandholm, 1995). Fore-strip (approximately 10ml) should be discarded in order to remove the normal bacterial flora from the teat canal and teat orifice to minimize contamination from these sources (Sandholm, 1995).

Samples for SCC determination need not be collected aseptically, however it is advised to sample from a clean teat, as faecal particles can influence the electronic counting methods used in diagnosis (Blowey, 2010).

Samples for bacterial culture are obtained in a sterile manner. The following guidelines taken from “Mastitis control in dairy herds”, by R. Blowey describe how one should obtain a sterile sample.

- 1) Wash and dry the teat with a paper towel.
- 2) Discard three squirts of foremilk from each quarter.
- 3) Coat the teat with a pre or post dip.
- 4) Apply gloves.
- 5) Scrub teat ends with cotton wool soaked in surgical spirit.
- 6) Open the sterile sample bottle, hold at 45° and apply a stream of milk making sure not to contact the teat end. Replace the lid.
- 7) Label each sample bottle with cow number and quarter.
- 8) Freeze or send to the lab, keeping cool if possible.

5. Clinical examination

- A correct clinical examination is of utmost importance when diagnosing clinical mastitis.
- A revision of the cow's **history** should also be taken into account.
- Owner: are the farmer's management skills putting the cows at risk of infection?
- Age: mastitis increases with age (Sandholm, 1995).
- Stage of lactation: for example *Coliform* mastitis is common near calving. *Streptococcal* and summer mastitis often occur in dry cows (Sandholm, 1995).
- Time of year: for example summer mastitis is prevalent in the fly season.
- Husbandry: are milking equipment and environmental conditions of appropriate standard and adequate level of hygiene to decrease the risk of contagious and environmental pathogen infection? (Sandholm, 1995).

It is worth reviewing the cow's previous use of antimicrobials and the farmer's teat dipping process to complete the review of history (Sandholm, 1995).

An accurate **clinical exam** takes into account the *general symptoms* exhibited by the cow. The major factors to consider during a clinical examination are as follows: if the cow is recumbent, if there is diarrhoea, if there is a decrease in rumen motility, lack of appetite or fever, increased heart rate and increased respiration rate (Sandholm, 1995).

The *udder* is the main point of interest. It is important to inspect the udder form and symmetry. Enlarged supra-mammary lymph nodes are a useful indication of infection. The udder skin and teat apices should be examined for injuries that could predispose for bacterial intra-mammary infection. The udder, supra-mammary area and teat should all be palpated. Hot, swollen, fibrous findings can all indicate intra-mammary infection (Sandholm, 1995).

Milk evaluation should also be taken into account. Poor or dispersed milk flow indicates a chronic teat injury. Clots, flakes and serous or purulent like appearance and a foul odour are positive indicators of mastitis (Sandholm, 1995).

As previously mentioned the physical changes in milk induced by mastitis can all be evaluated cow-side by examining the colour, the integrity, the rancid odour and the salty taste imparted on the sample.

The findings of a clinical examination usually result in either normal or infected quarters.

Normal quarter: milk and quarter look and feel normal (Faull, 1991).

Sub-clinical quarter: milk is contaminated with pathogens, however looks and feels normal (Faull, 1991).

As there is no way of distinguishing a normal from a sub-clinically infected quarter from visual inspection and palpation, a cow-side test capable of sub-clinical case detection is essential.

Clinical mastitis can be classified according to grades (Faull, 1991).

- Grade 1: the milk looks abnormal (clots) has a marked increase in inflammatory cells. The milk is likely to be infected with pathogens. The quarter feels normal and the cow is not systemically ill.
- Grade 2: the quarter looks and feels abnormal but the cow is not systemically ill.
- 2A: the quarter is swollen, hot and painful and sometimes discoloured. This can be an indication of acute mastitis.
- 2C: the quarter is hard and lumpy and not painful. It may be enlarged or contracted. This can be an indication of chronic mastitis.
- Grade 3: the quarter has acute mastitis and the cow is systemically ill.

6. Methods of mastitis diagnosis using Somatic cell count (SCC)

6.1 Conventional methods

California Mastitis Test (CMT)

Theory

The California Mastitis Test is a useful and simple cow-side test that both herdsman and veterinarian can use to diagnose mastitis (Lam, 2009). Its main function is to indirectly measure SCC by estimating the DNA content in milk (Viguier, 2009). The mechanism of action is based on Na-lauryl sulphate that dissolves the cell membranes (Sandholm, 1995). Consequently DNA is released and it forms a transient gel with the detergent. The viscosity of the gel is proportional to the leukocyte number (Viguier, 2009).

Method

Each kit comes with a 4-cup test paddle, a bottle of CMT concentrate, directions and a colour chart (Sandholm, 1995). **Figure 4** is an accurate description of the CMT in action.



Figure 4. The upper right milk sample is from an infected quarter, where the increased SCC coagulates with the reagent (Sandholm, 1995).

Equal volumes (2-3ml) of milk and Na-lauryl sulphate (CMT concentrate) are mixed on the test plate. The test plate has wells reserved for each quarter. The formation of viscous gel is evaluated as the plate is rotated. The result is evaluated after five seconds (Sandholm, 1995). The viscosity of the sample is positively correlated with the leukocyte number in the sample (Viguier, 2009).

Most manufacturers have included a pH indicator such as bromocresol purple in the reagent. Thus this test has two indicators: DNA quantification and pH indication.

Mastitis milk is more alkaline than normal milk and the gel turns purple in the presence of bromocresol purple (Sandholm, 1995). The intensity of the purple colour is positively proportional to the alkaline content of the milk sample.

As shown in **Table 1**, results can be scored on a scale of 1 to 5 using the Scandinavian scoring system, or results can be interpreted from negative to positive range using the U.S. system (Sandholm, 1995).

It is worth mentioning the durability of the test. Foreign materials do not interfere with the test and environmental temperature change has little effect on the test as long as the milk has been refrigerated and isn't over 2 days old (Duane, 1981).

The following excerpt taken from "Diagnostic value of the CMT in comparison to electronically counted somatic cells in bovine milk" by R. Redetzky (2005), describes the recommended procedures for the CMT.

1. Discard the first jets of milk into a strip cup.
2. Use only foremilk obtained after milk is completely ejected.
3. Add an equal amount of CMT reagent to the test milk in the cup.
4. Mix the milk with the reagent by gently swaying the paddle.
5. Decant supernatant reagent-milk mix carefully to a volume of 2ml.
6. Take readings within 20 seconds under good lighting conditions.
7. Use only sufficiently experienced operators.
8. Consider CMT scores: negative = no precipitates, or positive = precipitates.

Experimental findings

Findings from “Diagnostic value of the CMT in comparison to electronically counted somatic cells in bovine milk” by Redetzky (2005) show a linear correlation between the CMT results and SCC. The spearman’s coefficient of rank correlation was high ($r_s = .801$; $CI_p = .99 = .756, .846$).

In this article Redetzky states how it is “ preferable to evaluate CMT on the basis of comparisons with SCC as the gold standard”.

Just under 200’000 cells/ml for a negative CMT and up to 500’000 cells/ml for trace results of the CMT have been frequently been reported by authors (Sandholm, 1995; Redetzky 2005).

Table 1 portrays the classification of the CMT results, cell count variation and means within each class.

Scandinavian scoring system CMT class	Equivalent U.S. CMT score	Corresponding cell count range	Mean cell count
1	negative	< 200,000	100,000
2	trace (suspicious)	150,000–500,000	300,000
3	1 (suspicious)	400,000–1,500,000	900,000
4	2 (positive)	800,000–5,000,000	2,700,000
5	3 (positive)	> 5,000,000	8,100,000

Table1. Mastitis classification (Sandholm, 1995).

It has been reported that false positive reactions occur frequently on cows that have been fresh for less than 10 days, or on cows that are nearly dry. These animals should be tested closer to the middle of lactation. Cases of acute clinical mastitis milk have been shown to score a negative CMT. This is a consequence of leukocyte destruction resulting from bacterial toxins (Duane, 1981).

Summary

To summarise, it is fair to say that the CMT is practical, simple to perform and cost effective (US\$12 for 350 tests (Viguier, 2009)), however it is not as accurate as direct methods due to the fact that its results represent a range of leukocyte content other than an exact amount (Duane, 1981). The stage of lactation can also influence the CMT capability as described by Duane.

Commonly reported cell count levels in the range of up to 200'000 cells/ml for a negative CMT and of up to 500'000 cells/ml for trace results of the CMT (Redetzky, 2005; Sandholm, 1995).

It has been suggested that 100'000 – 200'0000 cells/ml indicates sub-clinical infection (Frundzyhan, 2008). Lams mention of healthy producing quarters are those that do not exceed the SCC of 70'000 cells/ml milk also supports this argument (Lam, 2009). So it is my belief that there is a small window (approximately 50'000 to 100'000 cells) where the CMT may fail to detect sub-clinically infected quarters.

N-acetyl-Beta-D-glucosaminidase (NAG-ase)

Overview

It has been proposed that LDH and NAG-ase have a common route with somatic cells produced as a result of mastitis (Chagunda, 2005).

“The prevalence of inflamed quarters is determined by assessing mastitis in lactating cows using one of the following methods: CMT, milk NAG-ase activity, or cell count” (Sandholm, 1995).

If NAG-ase is greater than 18 arbitrary units/l, the quarter is considered inflamed.

As described earlier, in the incidence of mastitis, enzymes connected with milk synthesis decrease and the enzymes associated with inflammation increase. Amongst these inflammatory enzymes is N-acetyl-Beta-D-glucosaminidase (NAG-ase).

NAG-ase is an intracellular, lysosomal enzyme, which is released into the milk compartment as the inflammation cells become activated or break down. During inflammation NAG-ase is released mainly from neutrophil granulocytes and cell lysis and to some degree from damaged epithelial cells (Pyorala, 2003).

A fluorometric NAG-ase assay based on micro-titration tray technology is a conventional method of mastitis detection developed in Finland (Sandholm, 1995). This concept is supported by over twenty years worth of literature (Pyorala, 2003).

The principle of the NAG-ase assay involves the mixture of the milk sample and the fluorogenic MUAG-substrate at pH 4.6 and subsequent incubation for 15 minutes. A hydrolytic reaction occurs. The fluorescence intensity is measured with a micro-titration tray reading fluorometer (Sandholm, 1995). This reaction is depicted in **Figure 5** below.

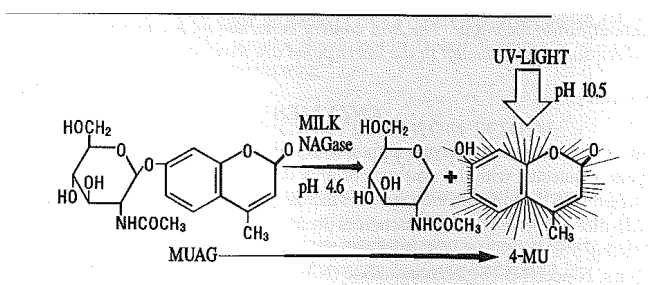


Figure 5. NAG-ase reaction (Sandholm, 1995).

The NAG-ase kit (Applied Diagnostics C. Finland) includes both a negative (normal milk) and a positive (masitic milk) control sample to ensure comparable results (Sandholm, 1995).

Additional features include automatic inter-quarter comparison: the quarter values are compared with the lowest quarter value of that cow (Sandholm, 1995). The NAG-ase assay is sensitive, reproducible and has a high throughput capacity (sandholm, 1995). According to Sandholm “The method has potential for monitoring the mastitis situation in herd. When analysing quarter milk samples form an entire herd, the program calculates both the percentage of inflamed quarters and cows”(Sandholm, 1995).

However over twenty years has passed and there is still no test commercially available today (Pyorala, 2003).

Experimental findings from “Changes in lactate dehydrogenase, N-acetyl-Beta-D-glucosaminidase, and SCC in relation to development of mastitis in dairy cows” (Chagunda, 2005), revealed that the activity of LDH and NAG-ase and the level of SCC in milk increased significantly with mastitis, suggesting that they could be useful in mastitis detection.

However there was a stronger correlation observed between LDH and SCC (.76), than NAG-ase and SCC (.58) in mastitic cows (Chagunda, 2005).

In a separate study the ability of NAG-ase activity in the detection of sub-clinical mastitis was evaluated. Using $> 400'000$ cells/ml as the threshold for infection, the test had an average of 17% false negatives and 2% false positive. Thus it was concluded that the NAG-ase test is a good indicator of sub-clinical mastitis.

6.2 Novel cow-side tests

PortaSCC®

Theory

A novel cow-side test for accurate SCC detection has been developed and can be used in three simple steps by both farmer and veterinary practitioner alike (Lam, 2009).

The PortaSCC® quick test is used onsite as a rapid cow-side test, for the detection of sub-clinical mastitis (Leslie, 2006).

A colour change in the test pad provided with this test correlates to the level of white blood cells in the milk sample (www.portacheck.com).

The principle is based on the enzymatic esterase reaction. White blood cells in the milk sample are trapped in the special reagent layer of the test pad, which also has a dye substrate immobilized on it. The enzyme esterase from the white blood cells will catalyze the colour dye reaction, generating a blue colour, which is proportional to the SCC in the sample (Viguiet, 2009).

The PortaSCC® test has been shown to have high sensitivity and specificity when SCC was used as the gold standard (Ken Leslie, 2003).

Method

The PortaSCC® test kit contains: test strips, activator solution, colour chart and pipettes. One can see these components in **Figure 6**.



Figure 6. Components of PortaSCC® quick test (www.portacheck.com)

Running the test (www.portacheck.com)

- Fore strip.
- Collect a milk sample into a clean and labelled container.
- Mix the sample.
- With a pipette place 4 drops of milk to the test strip sample well.
- Add 4 drops of activator solution to the sample well.
- Wait 5 minutes.
- Estimate the SCC by comparing the colour of the strip with the colour chart.
- Waiting longer to read the test strips can lead to false higher results.

Conditions of the test (www.portacheck.com)

- Store the kit at 2°C - 25°C (36°F - 77°F). Refrigerate, if possible.
- Store milk samples between 7°C and 35°C.
- Test in a shaded area, out of direct sunlight.
- The milk should be tested within 8 hours of sampling.
- If the milk is refrigerated, allow it to warm to room temperature before testing.
- Specific amount of milk sample is needed for the test to be accurate.
- Preservatives are not to be used as they interfere with the test result.
- Antibiotics will not interfere with the test.
- Inaccuracy can be observed when samples are not absorbed into the sample well.

Experimental findings

In an experiment conducted by Ken Leslie “An evaluation of the PortaSCC® test as a measure of udder health status in dairy cows”, the efficacy of the PortaSCC® quick test was evaluated.

615 milk samples were obtained from the individual quarters of cows in various stages of lactation and evaluated with the PortaSCC® quick test and a Bentley Soma Count 300. The SCC cut off point that defined disease was > 200, 000 cells/ml (Leslie, 2006).

Following the manufacturers guidelines, a single-use pipette was used to place one or two drops of the milk sample onto the absorptive area of the test. Four drops of wash solution were individually placed and absorbed into the test area. The colour of the test area was observed and recorded using a colour chart (Leslie, 2006).

When no colour change was seen the result was considered either negative, or zero.

If a light blue colour was observed, the result was recorded as ‘one’.

If the test area changed to an aqua colour the result was considered ‘two’.

A colour change corresponding to royal blue was recorded as ‘three’.

The recorded values of one, two, and three are used to correlate with a SCC value of 200,000 cells/ml, 500,000 cells/ml and 1,000,000 cells/ml.

Any reaction greater than or equal to one was considered a positive test result. Any change in colour of the test pad (indicating a cell count > 200, 000 cells/ml) was considered a positive PortaSCC® result.

The findings of this experiment highlight the accuracy of the PortaSCC® test. The Sensitivity was 74%, specificity of 94%, negative predictive value of 93% and a positive predictive value of 79%. The Spearman rank association test revealed the electronically counted SCC and the PortaSCC® to be highly correlated ($R^2 = .63$, $P = <0.001$ (Leslie, 2006)).

In another study, by Professor David Lee, the PortaSCC® is evaluated against electronically counted somatic cells of two separate laboratories (www.portachek.com). The samples were collected at random from Holstein cows at different stages of lactation. The results from the PortaSCC® were read with a digital reader as opposed to a colour chart. The digital reader calculates the actual cell count according to the depth of the blue colour on the test pad.

One can see the outlying points portrayed in the **Figure 7**. This was due to the thick or clumpy milk that failed to absorb evenly onto the test strip (www.portacheck.com).

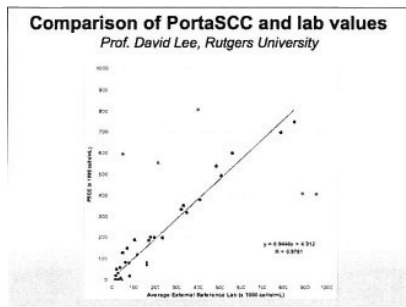


Figure 7, depicts the findings from the PortaSCC® and laboratory results.

(www.portacheck.com)

An excellent correlation of 98% was found between the PortaSCC® data and the average of the two reference labs (www.portacheck.com).

PortaCheck incorporated has compiled results from various countries regarding the PortaSCC® testing ability in detection of sub-clinical cases.

Excellent results were achieved. A high correlation of .88 was established between the PortaSCC® and the gold standard. A sensitivity of 92% and a specificity of 85% were found when a threshold of 200'000 cells/ml was used. These findings highlight the PortaSCC® excellent accuracy in sub-clinical case detection.

Professor Ruegg evaluated the efficacy of the PortaSCC® using a digital test reader against the CMT. Findings demonstrate that the PortaSCC® test is superior to the CMT in terms of accuracy, as a higher agreement was found between the PortaSCC® and the gold standard (www.portacheck.com). With a threshold of 200'000 cells/ml, the agreement between the PortaSCC® and laboratory gold standard was 87.8% (www.portacheck.com).

One can see the results in **Table 2**.

**Comparison of PortaSCC®, laboratory
and CMT values***

Prof. Pamela Ruegg, University of Wisconsin

CMT	No. of samples	PortaSCC® (cells/mL)	Laboratory (cells/mL)
Negative	147	107,844	104,687
Trace	12	486,583	386,500
One	13	406,462	481,462
Two	13	1,705,000	1,633,000
Three	16	1,988,000	2,328,000

* Association between Results of PortaSCC®, the CMT and Isolation of Mastitis Pathogens by Talita Amara and Pamela L. Ruegg, University of Wisconsin

Table 2. Findings from the PortaSCC® and the CMT (www.portacheck.com).

Feedback

Ireland, with a large cattle population having a concomitant importance in the economy, has been chosen to survey, and user satisfaction with the product has been gathered from farming enterprises abroad.

In a survey conducted by Ireland's only supplier (Duggan Veterinary Suppliers), the following data concerning the PortaSCC® quick test was gathered:

- Time on the market: August 2012.
- It has been tested and recommended by the two largest Irish dairy production companies (Dairy Gold and Glan Bia).
- Amount of product sold: 150 boxes per month (each box contains 40 test strips). Such is the success of the product that they have announced their intention to sell on-line, through their website (animalpharmacie.ie), in the near future. The quantity of the product sold in such a small niche market attests to its popularity.
- The price is extremely attractive: €44 to veterinarians, and €75 to farmers per box.
- PortaSCC® is the only product sold for early mastitis detection at this establishment, and as such occupies a unique position in the Irish market.

There has also been a very positive response to the capability of the product worldwide from customer feedback portrayed on the following page.



the
PortaSCC[®]
milk test

Customer Testimonials

"The PortaSCC[®] milk test is the greatest thing I have found in 12 years! I use the PortaSCC[®] milk test to pinpoint problem quarters. Anyone who has cows has problems at one time or another."

Cletus Eash, an organic dairy producer from Indiana, uses the PortaSCC[®] milk test to check fresh and treated cows, and to retest cows with high DHIA results. He tests his cows with the PortaSCC[®] milk test routinely, resulting in a bulk tank reading around 150,000.



Rachwal Brothers

"Excellent product! The PortaSCC[®] milk test is very easy to use and understand. It is fast and simple. No more guessing what the somatic cell count is."

The Rachwal Brothers of Weyauwega, Wisconsin, have been milking cows for over 19 years since taking over the family business started in 1951. They use the PortaSCC[®] milk test to check suspicious cows, re-test cows with high DHIA results, and to monitor treated cows.

"The PortaSCC[®] milk test is easy to use. It helps to identify suspicious cows and treat accordingly."

William Judge from Ontario, Canada, has been in the dairy business for over 50 years. He uses the PortaSCC[®] milk test as a routine mastitis practice to test cows at freshening, check suspicious cows and re-test cows with high DHIA results.

"We use it [the PortaSCC[®] milk test] to determine what cows to withhold from the bulk tank to keep the tank average low." Our current somatic cell count is under 100, 000, and the PortaSCC[®] milk test is one of the reasons."

Mr. Leon Corse is a certified organic farm owner from Whitingham, Vermont. His family has been in the dairy business for over 140 years. Mr. Corse currently owns 60 Holsteins and uses the PortaSCC[®] milk test as a routine mastitis management practice by checking all fresh cows before putting the milk into the bulk tank.

"We checked questionable cows using the PortaSCC[®] milk test strips. We found the information to be reliable and the test easy to implement."

Clark Vilter of Four Winds Farm, Hartland, Wisconsin, has been raising and showing prize-winning cows for more than 30 years. With such highly marketable cattle, Mr. Vilter thinks it is extremely important to check for sub-clinical mastitis and catch it in its early stages.



"Because we trust the PortaSCC[®] milk test, we test every cow when she freshens. If our cell count starts to get high, we check the suspected cows again."

Anna Fritz, the owner of Fritz Dairy Farm in Minerva, Ohio who has been in dairy business for over 50 years. She has been using the PortaSCC[®] milk test to test her herd for early signs of sub-clinical mastitis. She tests selected cows weekly to keep the somatic cell count at the lowest level.

Summary

To summarise the efficacy of the PortaSCC®, Leslie's experiment "An evaluation of the PortaSCC® test as a measure of udder health status in dairy cows", showed the PortaSCC® to have a high Specificity of 94%. However a sensitivity of only 76% was found, but this was due to clinical case samples not being absorbed onto test area correctly (Leslie, 2006).

The Spearman rank association correlation revealed that the electronically counted SCC and the PortaSCC® to be in strong agreement ($R^2 = .63$, $P = <0.001$).

Data from more recent experiments which evaluate the PortaSCC® test with a digital reader include: Professor Lee's study which showed a high correlation between PortaSCC® and laboratory values (98%) and Rueggs experiment where the PortaSCC® is shown to be a more accurate onsite test than CMT, whilst in high agreement of 87.8% with the laboratory gold standard (www.portacheck.com).

The only main limitation that's affiliated with this test is its inaccurate readings due to improper absorption onto the test pad area (www.portacheck.com). Clinical mastitis cases in which milk is clumpy and clotted and thus inadequately absorbed can lead to false negative results (Leslie, 2006).

This product's inability to read a clinical case is a major limitation to its use. However in practical terms this would be overlooked in an on farm situation where clinical cases would be more obviously known to the farmer.

This product is reported to be growing in popularity in Ireland, with Duggan veterinary supplies selling from 150 to 200 boxes per month.

From the customer testimonials, it is fair to say that the PortaSCC® is an appropriate tool for farmers to monitor SCC at quarter level. Due to its user-friendly onsite application and accuracy, this product should do well on the market, and significantly reduce the level of mastitis on the farm.

Delaval cell counter

Overview

In addition to the PortaSCC®, the Delaval cell counter is an accurate on farm method of estimating SCC (Lam, 2009).

The DeLaval cell counter is a portable device that can be performed at the cow's side, and has become a useful tool for managing milk quality (Blowey, 2010).

A milk sample is loaded into a cassette, inserted into the machine, and the results are obtained in under one minute (www.delaval.com).

The Delaval cell counter operates on the principle of optical fluorescence. Propidium iodide is used to stain nuclear DNA to estimate the SCC in milk (Viguier, 2009), and then a digital camera records the stained cell nuclei numbers, before sending the results to the management software system, where the results are displayed in less than one minute (www.delaval.com).

“Advantages: rapid test and the device is easily transportable. Disadvantages: relatively expensive” (Viguier, 2009). The cost is currently €2'800 (Ft 789'178 (taurina.hu)).

In a study by Ruegg “ Evaluation of an on farm test to estimate SCC”, the Delaval cell counting method was tested against the gold standard of cytological SCC. Positive findings were yielded from this study. The correlation between the SCC and the electronic cell count of somatic cells was .92 and κ coefficient of .82.

With a threshold of 205'000cells/ml, the sensitivity and specificity for detection of intra mammary infection was 91.3% and 96%. It is thus established that the Delaval cell counter shows acceptable ability to detect SCC on quarter milk samples.



Figure 8. Delaval electronic cell counter. (www.delaval.com)

6.3 Future Prospects

1) Lactate dehydrogenase (LDH) measurements as an indication of mastitis.

LDH is positively correlated with SCC, but with the benefit of not being as easily affected by factors such as stress, nutrition, parity and stage of lactation (Chagunda, 2005).

Measuring the levels of inflammatory related enzymes is a useful approach in the detection of mastitis (Viguier, 2009).

Hiss conducted an experiment to test this concept with the use of dry chemistry and a portable spectrophotometer to measure LDH activity and then evaluated these results against the gold standard (Hiss, 2007).

The standard at which sub-clinical mastitis was defined was in accordance with the requirements of the German Veterinary Society.

SCC calculation was done using a Fossomatic cell counter in which the threshold for sub-clinical infection would be a SCC > 100'000 cells/ml and bacterial culture positive in two out of three weekly samples.

LDH activity was determined spectrophotometrically. The methods adopted by Hiss in this study have resulted in a major scientific break through, for e.g., *raw milk samples* were collected and evaluated. This eliminates the laborious effect of pre-treating and preservation the samples.

Also, *on site* equipment (portable spectrophotometer and DT 60 II) was used to measure the LDH activity.

Positive findings were yielded from this study. From **Table 3** one can see that the LDH activity was low in foremilk samples in which no bacterial growth could be observed (median; 89 U/l). Samples being positive for growth of pathogens exhibit a higher LDH activity compared to samples without bacterial activity.

The SCC gold standard and LDH were positively correlated ($r = .76$ (Hiss, 2007)).

	<i>n</i>	LDH (U/l) Median (range)	<i>p</i> ¹	<i>p</i> ²	Hp (µg/ml) Median (range)	<i>p</i> ¹	<i>p</i> ²	SCC (10 ³ /ml) Median (range)
Sterile	79	89 (75–144)		0.001	0.7 (0.35–16.0)		0.001	40 (4–1 512)
<i>C. bovis</i>	70	92 (75–245)	n.s.	0.001	1.85 (0.35–85.0)	0.001	0.001	70.5 (6–2 249)
Mixed infection	45	104 (72–700)	0.001	0.001	2.4 (0.5–150.2)	0.001	0.001	136 (12–10 000)
CNS	60	98 (73–700)	0.006	0.001	3.1 (0.35–576.0)	0.001	0.001	167 (9–4 171)
<i>Streptococcus</i> spp.	29	246 (76–700)	0.001	n.s.	4.4 (0.5–974.0)	0.001	0.001	405 (32–10 000)
Mixed infection + <i>S. aureus</i>	35	103 (74–165)	0.006	0.001	4.8 (0.35–737.4)	0.001	0.001	335 (8–8 804)
<i>S. aureus</i>	49	167 (82–680)	0.001		39.6 (0.35–304.8)	0.001		1,741 (16–10 000)
<i>E. coli</i>	3	284 (175–396)	not tested	not tested	81.0 (59.0–184.0)	not tested	not tested	8 861 (1,658–10 000)

Table 3. LDH activities of selected udder quarters (Hiss, 2007).

As one can see from **Table 4**, the sensitivity and specificity were above 81% for LDH, when a threshold of 100'000 cells/ml was used.

	Cut-off	Sensitivity (%)	Specificity (%)	Youden Index
Hp (SCC 100 000 cells/ml)	2.2 µg/ml	85	92	0.77
Hp (SCC 200 000 cells/ml)	2.7 µg/ml	89	92	0.81
LDH (SCC 100 000 cells/ml)	99.5 U/l	81	86	0.67
LDH (SCC 200 000 cells/ml)	103.5 U/l	80	87	0.67

Table 4. Sensitivity and specificity of LDH (Hiss, 2007).

To summarise, from this experiment, LDH specificity was 86% and sensitivity 81% in sub-clinical case diagnosis. SCC and LDH were positively correlated and their relationship was evaluated using the Spearman rank correlation coefficient ($r = .76$).

In my opinion Hiss and co-workers “raised the bar” by onsite analysis using dry chemistry and a portable spectrophotometer and had comparable variation coefficients to the assays performed in the lab environment.

I think LDH as an indication of mastitis is useful in mastitis diagnosis and with onsite potential and high experimental accuracy would be a promising prospect for future on farm use.

2) Frundzhyans improved bioluminescent assay

Frundzhyans experiment “Improved bioluminescent assay of SCC in raw milk” is a new concept of mastitis detection. “ Owing to its sensitivity, specificity and simplicity it has potential for onsite use for mastitis diagnosis” (Frundzyhan, 2008).

The assay is based on the principle: ATP is consumed and light is emitted when firefly luciferase catalysis oxidation of D-luciferin (Sigma-Aldrich).

Frundzhyans concept to use a bioluminescent assay to determine the total non-bacterial ATP in milk $[ATP_{\Sigma}]$ is evaluated against previous methods, where SCC determination was estimated using a bioluminescent assay for the determination of ATP from somatic cells $[ATP_{som}]$.

From Frundzhyans experiment, results are as follows; a high correlation ($R^2 = .99$) was found between the measured bioluminescence and SCC by direct microscopy. The observed detection limit of the bioluminescent milk SCC assay was as low as 900 cell/ml. Time of analysis was 2-3min per sample (Frundzyhan, 2008).

As regards the previous method, the results portray a positive correlation between the bioluminescent assay of ATP of somatic cells and that of direct microscopy ($R^2 = .95$), however this is not quite as accurate as the assay undertaken by Frundzyhan. This assay is therefore an improvement on the previous concept.

Table 5 shows the values of SCC and ATP concentration in milk samples, where the positive correlated results exist between $[ATP_{\Sigma}]$ and direct SCC.

1	2	3	4	5	SCC $\times 10^{-5}$, cell/ml,	
					6	7
	SCC $\times 10^{-5}$, cell/ml†	$[ATP_{\Sigma}]$, nmol/ml	$[ATP_{exp}]$, nmol/ml	$[ATP_{som}]$, nmol/ml	calculated by the value:	
1.	0.8	0.19±0.01	0.11±0.01	0.08±0.04	1.2	0.8
2.	1.0	0.21±0.01	0.10±0.01	0.11±0.01	1.4	0.9
3.	1.1	0.25±0.02	0.22±0.02	0.03±0.01	0.7	1.0
4.	1.3	0.25±0.04	0.21±0.02	0.04±0.01	0.8	1.0
5.	1.4	0.28±0.05	0.14±0.05	0.14±0.06	1.6	1.1
6.	1.6	0.40±0.02	0.19±0.04	0.21±0.04	2.0	1.5
7.	1.6	0.62±0.04	0.40±0.04	0.24±0.05	2.2	2.2
8.	1.9	0.40±0.02	0.13±0.03	0.27±0.04	2.3	1.5
9.	2.3	0.63±0.08	0.30±0.05	0.33±0.05	2.6	2.2
10.	2.3	0.80±0.04	0.12±0.04	0.68±0.05	3.9	2.7
11.	2.8	0.83±0.11	0.09±0.03	0.74±0.11	4.1	2.8
12.	3.7	1.20±0.10	0.10±0.04	1.10±0.10	5.2	3.8
13.	4.7	1.41±0.10	0.11±0.03	1.30±0.10	5.7	4.3
14.	5.0	1.80±0.51	0.10±0.02	1.70±0.70	6.6	5.3
15.	5.7	1.80±0.11	0.10±0.03	1.70±0.10	6.6	5.3
16.	10.0	2.80±0.30	0.20±0.05	2.60±0.30	8.4	7.8
17.	10.0	3.60±0.20	0.10±0.03	3.50±0.20	10.0	9.6
18.	11.1	3.30±0.50	0.20±0.03	3.10±0.10	9.3	8.9
19.	16.0	5.70±0.40	1.10±0.04	4.60±0.60	11.7	14.2
20.	22.0	9.01±0.21	0.60±0.02	8.41±0.21	17.1	19.8

† Measured by direct microscopy cell count, so did not exceed 15% of the mean in all samples (not shown)
‡ Calculated by Equation (5)
§ Calculated by Equation (6)

Table 5. SCC and ATP concentrations in milk samples (Frundzyhan, 2008).

7. Cow-side methods of mastitis diagnosis using Electrical conductivity (EC)

1) Milking unit

“The principle of measuring electrical conductivity of milk. Electric current flows more easily through mastitic milk, than normal milk because the electrolyte content is higher and fat content lower” (Sandholm, 1995).

The basic principle of the milking machine involves the application of a vacuum to the teat end that causes the teat canal to open and the milk to flow out. The oxytocin induced letdown reflex contributes to this process (Blowey, 2010). The main aim is the swift removal of milk, with minimal damage to the udder (Blowey, 2010).

Electrical conductivity (EC) meters can be attached to the milking units (Sandholm, 1995). The milking unit consists of four teat cups, the claw, the pulsator and connecting tubes (Blowey, 2010).

The conductivity is measured from each quarter and then each quarter result is compared with one another (Sandholm, 1995).

However, there are variations in conductivity levels during the milking and even non-mastitis related variations in EC could be problematic (Viguiet, 2009).

The major problem with using conductivity to detect mastitis, is that there is a large inter-cow variation and the conductivity changes during milking (Sandholm, 1995).

From **Figure 9**, one can see how the conductivity changes during milking.

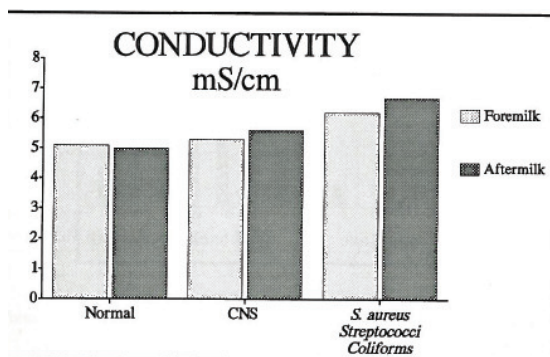


Fig 9. The relationship between bacterial infection and the conductivity differences between foremilk and after milk samples. (Sandholm, 1995).

2) The milking robot

The milking robot is a recent development. It was first installed in the Netherlands in 1992 (Blowey, 2010). The principle of the robot is identical to that of the milking machine, apart from the fact that the robot has to attach the machine automatically (Blowey, 2010).

Most robots are equipped with electrical conductivity meters that can help with the early detection of mastitis (Blowey, 2010).

Robotic milking has become quite popular in recent years (Viguier, 2009). It provides an “ideal format for online mastitis monitoring” (Viguier, 2009).

The robot as a support in mastitis diagnoses has adopted milk colour analysis, in addition to EC. It has been stated that EC on its own is not a reliable enough parameter for a correct diagnosis (Viguier, 2009).

For this reason major manufacturing companies such as Lely and Delaval, measure other parameters in addition to electrical conductivity for a more accurate result.

Both of these companies produce milking robots with a unique milk quality control system located in the robotic arm.

The Lely Astronaut A4 milk quality control measures at quarter level: EC, milk colour, milking times and temperature (www.lely.com).

The Delaval voluntary milking system (VMS) milk quality control system is composed of four optical, quarter milk meters, which monitor alterations in the flow rate, milk yield and EC. Abnormal milk can then be diverted automatically away from the main tank and the management software records all received data. (www.delaval.com).

In addition to the milk quality control, there is an accessory available for the Delaval VMS. This is the online cell counter (OCC). It is a device that measures the SCC of cows at every milking. A detergent (triton- x) breaks down the cell wall, followed by staining of the nuclei by propidium iodine. A digital camera records these stained images in which the results are compiled and then sent to the management software system. The OCC is expensive €7'400 (www.delaval.com). But according to a cost benefit analysis undertaken by DeLaval. The benefits outweigh the costs.

3) Experimental findings

In a study by Kasikci “Relations between EC, SCC, CMT and some quality parameters in the diagnosis of sub-clinical mastitis in dairy cows” (Kasikci, 2012) : EC, SCC and CMT were all evaluated. A positive correlation was found between CMT, and EC ($P = < .001$).

It was concluded that EC was positively correlated with the CMT and SCC, nevertheless its reliability would be further enhanced if other parameters were measured also (Kasikci, 2012).

In a separate study “ Mastitis detection based on electric conductivity of milk” (Janzekovic, 2009), the average measurement of milk samples from four quarters was tested with a microprocessor controlled device (Mastitron LF 3000) and evaluated against SCC using cytological examination and the CMT.

The results are as follows: when the EC was higher than the threshold level of 6.5mS/cm, it was positively correlated with the corresponding high SCC. A positive correlation was found between ECM and SCC, and EC and CMT in this study ($P \leq .01$). The reliability of the EC measurement was 80% and was found to be largely dependant on alteration of the milk composition (Janzekovic, 2009).

As regards the efficacy of milking robots ability to detect mastitis. An experiment “Effectiveness in SCC determination in the milking robot” (Lusis, 2010) was carried out to evaluate the Delaval voluntary milking system with that of SCC gold standard. According to Lusis “ results indicate that the milking robot online cell counter is an effective tool to observe continuously the SCC, and has proven to accurately find cows with $>200'000$ cells/ml of milk”. The milking robot has thus proven itself effective in the detection of mastitis.

8. Discussion

“Healthy udders are economically profitable” (Lam, 2009). For this reason effective mastitis diagnosis is essential.

From researching this topic, it is evident that there is sufficient knowledge in the field of mastitis diagnostics available, with more and more modern methods being introduced (Lam, 2009).

Traditional methods of mastitis detection include estimation of SCC, measurement of biomarkers such as NAG-ase and LDH, and bacterial culturing methods (Viguier, 2009).

More than twenty years of research supports the concept of NAG-ase as a suitable indicator of mastitis.

A fluorometric NAG-ase assay has been developed, researched and shown to have good results, however there is not as yet any test commercially available today (Pyorala, 2003).

Evidence from an experiment by Chagunda reveals that NAG-ase and SCC are positively correlated, however there is a stronger agreement between LDH and SCC (Chagunda, 2005).

This effectively leads one to believe that LDH is in fact a better indicator of mastitis.

One of the most traditional cow-side tests is that of the physical examination. Physical changes in the milk composition induced by mastitis, can help identify infected quarters.

For e.g., increased lipase can impart a rancid flavour to the milk (Blowey, 2010), and the increase of sodium and chloride ions give the milk a salty taste. A decrease in milk yield is also an important indication of mastitis (Sandholm, 1995).

The CMT, PortaSCC® and the Delaval cell counter, are all common cow-side methods, which use SCC to identify infected quarters (Viguier, 2009). These methods have an advantage over electronic somatic cell counters, as they can accurately pin point the affected quarter, as opposed to giving an evaluation of the whole udder (Blowey, 2010).

Evidence suggests that these three cow-sides are relatively accurate, user-friendly and with the exception of the Delaval cell counter, cost efficient.

When Redetzky evaluated the CMT, a linear correlation between CMT results and the SCC gold standard was calculated using Spearman’s coefficient of rank correlation. However although it may to some degree be accurate, the threshold level at which it detects infection is worrying.

Just under 200’000 cells/ml for a negative result and up to 500’000 cells/ml for a trace result, has been reported in many incidences (Sandholm, 1995, Redetzky, 2005).

One author claims that changes seen with this test are only noticed above 400'000 cells/ml (Blowey, 2010). False positives can be a frequent finding with fresh cows (Duane, 1981), variations in results have been reported and it can be unable to pick up infection in some quarters (Blowey, 2010).

This evidence implies that there are a lot of inaccuracies associated with this test.

The spearman rank association test revealed a positive correlation between the SCC gold standard and the PortaSCC® ($R^2 = .63$, $P = < .001$), in an experiment to identify clinical and sub-clinical infected quarters (Leslie, 2006).

An Excellent correlation (98%) has been found between the PortaSCC® results and the average of two reference labs (www.portacheck.com).

In a study by Ruegg a higher agreement between PortaSCC® and the laboratory SCC gold standard was established than that of the CMT and the laboratory gold standard (www.portacheck.com). These findings lead one to believe that the PortaSCC® test is in fact more accurate than the CMT.

However inaccuracy due to improper absorption on to the sample test pad is a limitation that can lead to increased numbers of false negatives.

From Leslie's experiment it was found that most clinical mastitis cases were improperly absorbed due to their thick and clumpy structure.

In an on farm practical situation this wouldn't necessarily be an issue, if the farmer would be observant and have the potential to single out these clinical cases.

From Professor Lee and Ruegg's experiments, the PortaSCC® has yielded excellent results with respect to the gold standard used by the laboratories. I would definitely recommend this test for a farm with a high incidence of sub-clinical cases, due to its attractive price and accuracy.

From experimental findings the Delaval cell counter seems to yield very accurate results. When evaluated against the SCC gold standard a positive correlation of .92 was found and a sensitivity and specificity was greater than 91% when a threshold of 205'000 cells/ml were used (Ruegg, 2009). However this device is relatively expensive, approximately 789'178 Ft (€2'800 (taurina.hu)).

The prospective tests reviewed in this thesis show promising cow side potential, especially the experiment conducted by Hiss.

With the use of dry chemistry and a portable spectrophotometer extremely accurate results were obtained. LDH and the SCC gold standard were positively correlated ($r = .76$). The

sensitivity and specificity was greater than 81% when a threshold level of 100'000 cells/ml was used. This is a promising prospect for the future.

The fact that LDH positively correlates with SCC but isn't as easily affected by conditions such as stress, nutrition, stage of lactation etc, imply that it has the potential to be a more accurate onsite diagnostic method than those which use SCC alone. LDH levels also rise earlier than SCC, making it an excellent marker for early detection (Chagunda, 2005).

These two key points highlight the importance and superiority of LDH measurement to SCC in mastitis diagnosis.

Frundzyhans method of detecting mastitis with a bioluminescent assay to determine [ATP Σ] proved to be very accurate ($R^2 = .99$) and with an ability to detect cells as low as 900cell/ml. However this experiment is not as close to becoming an onsite cow-side test as the experiment conducted by Hiss.

“Any on-line mastitis detection is currently performed using EC, SCC or colour determination, with milk EC being the most commonly used ‘on-line’ test” (Viguier, 2009). However frequent reports claim that EC testing alone is not a reliable enough method of mastitis diagnosis (Viguier, 2009; Kasikci 2012).

Modern conductivity meters can be attached to the milking units, however inter-cow variations and conductivity changes are known to occur (Sandholm, 1995), making this a less likely method of accurate mastitis diagnosis.

Milking robots have proven to be more successful in their diagnosis, and over 4 % of Dutch farmers now use this method. Other parameters are usually measured in addition to EC (Viguier, 2009).

The Delaval voluntary milking systems accuracy in detecting SCC greater than 200'000 cells/ml confirms its importance in detection of mastitis (Lusis, 2012). However the online cell counter (the accessory that improves the milk quality controls accuracy) is expensive.

Delaval manufacturers claim that the benefits of this device outweigh the costs that one would suffer without it (www.delaval.com).

9. Conclusion

The changes in milk induced by mastitis are the basis for an appropriate diagnosis. Physical changes detected from examining the milk can lead to an immediate diagnosis of a clinical case. Sub-clinical cases are difficult to detect due to the absence of visible signs, thus appropriate diagnostic tests are needed.

From reviewing traditional methods of mastitis detection, both inflammatory enzymes: LDH and NAG-ase increase with inflammation, however it can be affirmed that the activity of LDH is a more accurate indicator of sub-clinical mastitis than that of NAG-ase (Chagunda, 2005).

Cow-side tests are especially important in the diagnostic field and can be used by both farmer and veterinarian alike. Practicality with simplicity, accuracy and cost efficiency in mind is the main attraction to this method (Viguier, 2009).

Cow-side tests that utilize physical changes in milk samples, SCC alterations and/or EC are widely used in mastitis diagnostics.

It can be concluded, that cow-side tests that employ SCC for mastitis diagnosis are more accurate than ones that measure EC alone (Viguier, 2009). The described cow-side tests are all reasonably accurate and some are more cost effective than others.

For appropriate selection of any one cow-side test, I would recommend a revised cost benefit analysis program, to be envisaged by the producer.

If for e.g., there is a high incidence of mastitis on a given farm, I would then advise the Delaval cell counter or the PortaSCC®, the more accurate methods of detection. The Delaval cell counter is expensive, but an accurate and durable machine that has proven itself exceptionally well in the field of mastitis diagnosis. The PortaSCC® has also shown to be very accurate in diagnosing sub-clinical cases and comes at an affordable price.

Earlier detection and prevention minimizes the losses the farmer may encounter.

An ideal cow-side test to diagnose early cases would immensely improve the farm management system. There would be less culls, more milk production and thus an overall improved udder health status on the farm.

I am hopeful for what the future will bring. I believe LDH measurement as an indication of mastitis will become a more accurate onsite test than any of the previous mentioned cow-side tests that use SCC alone. The reason for this being that LDH levels rise earlier than SCC, and LDH measurements aren't influenced to the same degree by certain variable factors which

influence SCC (Chagunda, 2005), thus giving rise to an earlier and more accurate detection method.

“Continuous monitoring of mastitis, and its careful management, is essential for the well-being of a dairy herd” (Viguier, 2009).

10. Summary

Effective cow-side tests that can be used by farmers and veterinarians, have the potential to stop the propagation of mastitis in a herd.

An evaluation of conventional, novel and prospective cow-side tests was reviewed in this paper.

Each cow-side test was evaluated against a gold standard, and the results were compared with one another. Factors concerning 'onsite' practicality were also taken into account, in order to narrow down the best methods.

It is evident that the Delaval cell counter and PortaSCC® cow-side tests are amongst the most efficient cow-side tests in mastitis diagnosis. The Delaval cell counter showed a positive correlation (.92) with the SCC gold standard, and had a specificity and sensitivity greater than 91% when a threshold level of 205'000 cells/ml was used (Ruegg, 2009).

An excellent correlation of 98% was achieved when the PortaSCC® was evaluated against the average of two reference laboratories SCC gold standards (www.portacheck.com).

Future prospects, such as the ability to measure LDH activity onsite with dry chemistry and a portable spectrophotometer, while yielding great results (Hiss, 2007) 'raises the bar' for currently used cow-side tests.

In this review, mastitis diagnostics that are essential in performance monitoring are discussed. Recent advancements within this field, with particular emphasis on the cow-side approach are explored.

“ Monitoring udder health performance is impossible without reliable and affordable diagnostic methods” (Lam, 2009).

11. Acknowledgements

This literature review was carried out at the Department of Animal Hygiene, Faculty of Veterinary Medicine, Szent Istvan University, Budapest.

It is with immense gratitude that I acknowledge the support and help of my supervisor, Dr. Kovacs.

I am very appreciative of the support I had from the faculty librarians at Szent Istvan University. The library and computer facilities have been indispensable.

I wish to thank Professor Bryan Fanning at University College Dublin, Ireland, who was kind enough to send me specific journals and articles on mastitis related topics.

I am most grateful to Dr. Gerard Slattery, DVM, who provided me with the insight of mastitis diagnosis under practical circumstances.

I wish to thank Donnocha Duggan of *Duggan veterinary supplies* for his support and honesty and for taking part in a brief product survey. I would also like to thank Ayoma Finlay at PortaCeck incorporated who provided me with information on the PortaSCC® quick test.

I am very thankful to all who have supported me.

12. References

1. Viguier, C. 2009: Current trends and future prospectives. *Cell Press* p. 486-490.
2. Lam, TJGM. 2009: Mastitis diagnosis and performance monitoring: *Irish vet journal*. Vol 62.
3. Blowey, R. 2010: Mastitis control in dairy herds second edition, p. 15, 36 -55,60-74,154 -155.
4. Sandholm, M. 1995: The bovine udder and mastitis, p. 37, 59 – 60, 76 – 96, 105 – 111& 255.
5. Pyorala, S. 2003: Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.* 34, p 565-578.
6. Faull, W.B. 1991: A mastitis handbook for the dairy practitioner, p. 9 & 17.
7. Redetzky, R. 2005: Mastitis in dairy production. “Diagnostic value of the CMT in comparison to electronically counted somatic cells in bovine milk” p 487 – 493.
8. Chagunda, 2005: Mastitis in dairy production. “Changes in LDH, NAG-ase and SCC in relation to the development of mastitis in dairy cows.” p 445 – 448.
9. Chagunda, 2005: A model for the detection of individual mastitis based on an indicator measurement in milk. *J. dairy sci* (89) p 2980-2998.
10. Frundzyhan, VG. 2008: Improved bioluminescent assay of SCC in raw milk. *Journal of dairy science* 75, p. 279-283.
11. Hiss, Mueller. 2007: Haptoglobin and LDH measurements in milk for the identification of sub-clinically diseased udder quarters, *Vet. Med*52, p. 245-252.
12. Leslie, K. 2006: An evaluation of PortaSCC® test as a measure of udder health status in dairy cows, *University of Guelph paper* p. 2-5.
13. Ruegg, P. 2009: An evaluation of an on farm test to estimate SCC. *Journal of dairy sciences, volume 92, issue 3*, p. 990 - 995.
14. Kasikci, G. 2012: Relations between electrical conductivity, somatic cell count, California mastitis test and some quality parameters in the diagnosis of sub-clinical mastitis in dairy cows. *Turkish journal of animal science*, vol 36 (1), p. 49 – 55.
15. Janzekovic. M, 2009: Mastitis detection based on EC of milk. *Journal of achievements in materials and manufacturing engineering*, vol 34, issue 1, p. 39 – 46.
16. Lusi, I. 2010: Effectiveness of SCC determination in the milking robot, *Engineering for rural development*, p 112 – 116.

17. Porta Check, inc. www.portacheck.com, 2012. DL0079 Rev. A0
18. Duane, N: Using the California mastitis test to detect sub-clinical mastitis (1981).
Historical materials from University of Nebraska – Lincoln extension.
19. Paper 483, <http://digitalcommons.unl.edu/extensionhist/483>.
20. Kahn, Line. 2010: The Merck Veterinary manual *tenth edition* p 1248.
21. Delaval global: www.delaval.com
22. <http://www.delaval.com/en/-/Product-Information1/Milking/Products/Stallwork/VMS-station>.
23. http://www.icar.org/Documents/Verona_Presentations/DeLaval.pdf
24. http://www.lely.com/en/milking/robotic-milkingsystem/astronaut-a4/milk-quality_3
25. http://taurina.hu/en/termekek/kategoriak/szomatikus_sejtszamlalo/filter%7Clabel:21