

ON-LINE SENSORS FOR EARLIER, MORE RELIABLE MASTITIS DETECTION

D.S. Whyte, P.T. Johnstone, R.W. Claycomb and G.A. Mein

Sensortec, Hamilton, New Zealand

SUMMARY

The cause-effect spectrum allows a more complete understanding of the progression of bacterial infection in the mammary gland. By strategically identifying components in the spectrum that are of diagnostic benefit, on-line sensors will enable farmers to automatically detect the onset and progression of mastitis. In the future, these technologies will allow earlier, more reliable detection of sub-clinical and clinical infections.

INTRODUCTION

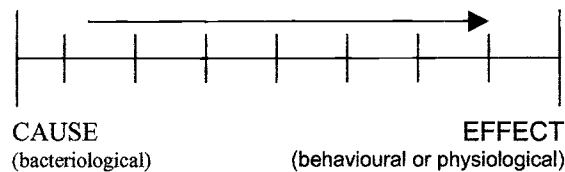
Most farmers, consultants, veterinarians and milk processing companies around the world have listed “mastitis detection” as the primary need for development of on-line sensing technology. However, when these groups of people were asked what exactly they wanted to detect and what they thought “mastitis” referred to, it became obvious that there exists a large amount of confusion regarding mastitis and mastitis detection.

This is because the true definition of mastitis is quite specific, while the term is often used to refer to a broad and complex sequence of events. “Mastitis” is defined by Webster and the International Dairy Federation as an “inflammation of the...udder.” If this is “true mastitis,” two interesting questions become obvious.

- How can you build a sensor that detects “inflammation?”
- Is “true mastitis” really what everyone wants to detect?

Each person has formulated their own definition of what they really want to detect when they use the phrase, “detecting mastitis.” Some define “detecting mastitis” as when a test of bacterial pathogens is positive, others when somatic cell count (SCC) reaches a particular level, others when clinical signs such as clotting of milk or swelling of the udder occur. An overall picture of mastitis can be described in a cause-effect spectrum (Figure 1).

Figure 1 Diagram of the Sensortec Cause-Effect Spectrum® (1)



We are working on a range of sensors covering the cause-effect spectrum. The rationale under-pinning the cause - effect spectrum is that the further away from the cause (bacterial infection) then the more likely a physiological effect is caused by something other than the infection. The following mastitis detection systems are being investigated:

- Lactate. Because bacteria produce lactate as they grow and multiply in the udder, lactate is one of the earliest direct indicators of bacterial infection.
- MAA. The cow's immune system's initial response to an infection is the so-called Acute Phase Response. Acute phase proteins, such as Milk Amyloid A (MAA) are produced by the cow's udder.
- SCC. Somatic cell count is very important for herd management for many farmers. We have a direct on-line SCC sensor.
- Conductivity. Conductivity is further along the cause-effect spectrum. It is a direct effect of tissue damage, but many other things apart from bacterial infection also affect conductivity. Nevertheless, it can be used to detect mastitis if all four quarters are measured throughout milking, and these data are compared with that cow's previous quarter conductivity data.

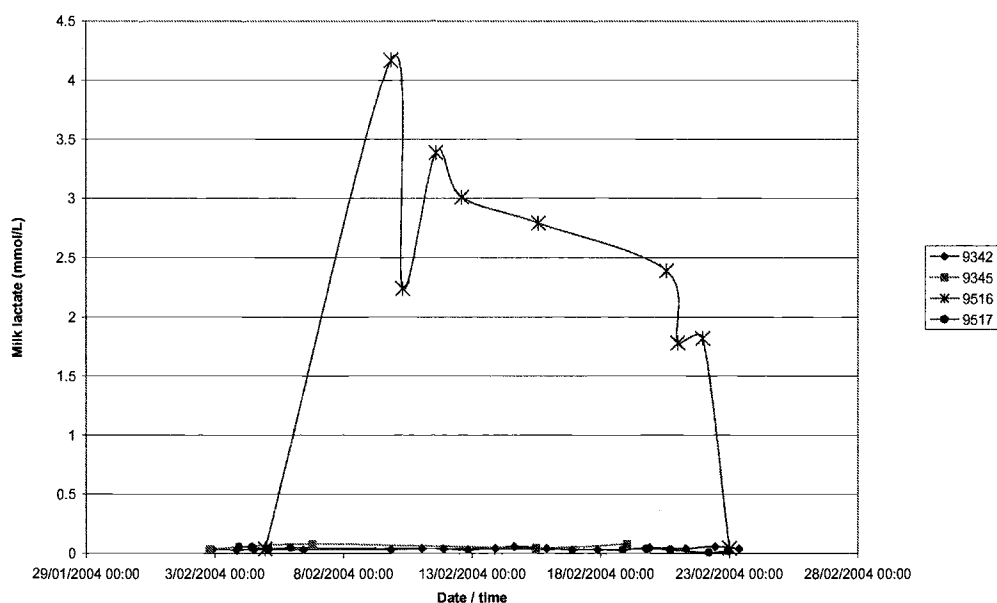
Lactate

The lactic acid (lactate) level in milk has been shown to be a good indicator of clinical and sub-clinical mastitis in dairy cows (2). During an infection the milk oxygen level decreases, leading to an anaerobic environment and the subsequent production of lactate. The on-line measurement of lactate thus provides useful information for herd management as an early indicator of an infection.

Sensortec has installed a commercially available L-lactate measuring device in an automated milking parlour for on - line analysis. One quarter was monitored during each milking of every cow over the period of one month. The data were matched to herd management software providing information on volume, conductivity, visual infections and treatments.

It was found that cows with high SCC and known infections had continually elevated levels of L-lactate. Lactate levels often varied widely between milkings indicating changes in infection status or severity. Figure 2 shows some typical lactate curves for one infected cow and three uninfected cows.

Figure 2 A cow that had an infection which has self-cured after an 18-day period



MAA

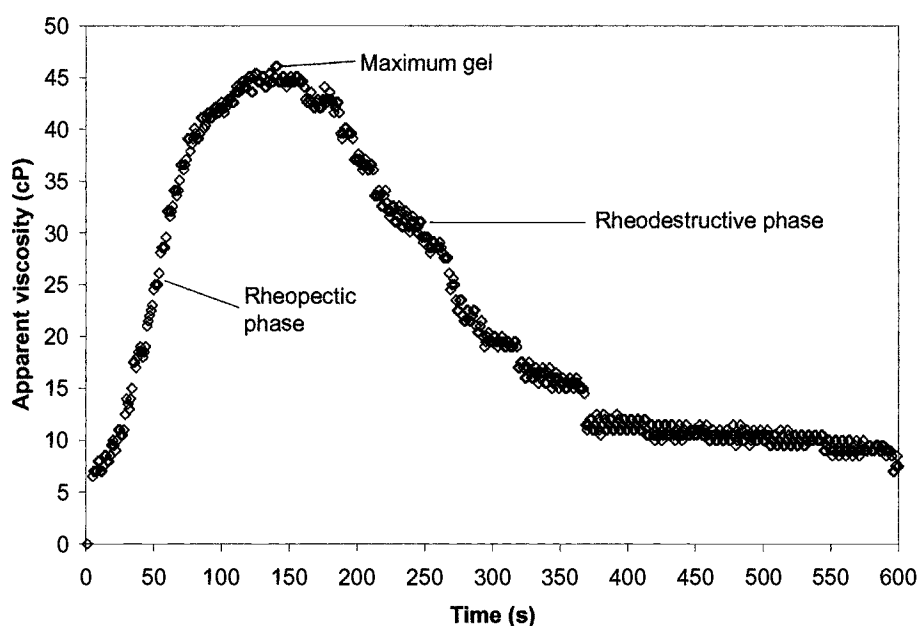
Acute phase proteins (APP) are produced by all mammals to varying degrees in an acute phase response (APR) to an episode of infection, trauma or disease. Production is induced by cytokines, as part of triggering the body's defense and repair mechanisms. APP used in monitoring animal health are Haptoglobin (Hp), Serum Amyloid A (SAA) and C-Reactive Protein (CRP), as they show the most pronounced and rapid rise in the majority of species.

Recently, amounts of SAA have been discovered in the milk of infected cows. It is thought that this is an isoform of the APP, SAA, namely MAA, and is synthesised in the udder (3). MAA is an APP, which helps trigger the immune system to respond to an antigen or challenge to the udder. This early signal may be in response to a bacterial infection or also physical damage and other stress. The levels of MAA in milk increase before SCC levels rise and may be useful as an early warning of infection.

SCC

A common off-line test for SCC is the California Mastitis Test (CMT). The CMT was developed in 1957 by Schalm and Noorlander who modified the Whiteside test (4). The test involves the addition of an anionic surfactant to the milk. The reagent interacts with the DNA and proteins in somatic cells to form a gel. The viscosity of the gel is then measured and calibrated against the somatic cell concentration. Figure 3 shows the apparent viscosity of the gel over time (5).

Figure 3 Illustration of the time dependency of detergent-DNA gel formation and degradation, showing gradual formation of gel (rheoplectic phase), maximum gel formation and the rheodestructive phase



We have undertaken extensive farmer consultations with groups in 7 countries and 11 markets, which illuminated the two likely major uses for an on-line SCC sensor. The first is early mastitis detection, when SCC begin to rise, which could prompt an immediate action to maintain optimal animal health. The other major use is bulk tank SCC management, where farmers can identify, remove or treat problem cows. Different markets were then interested in several measurement levels depending on the market's testing thresholds for bulk tank SCC. From this, we determined a need for flexible band reporting, rather than strictly quantitative results.

The SCC thresholds for this trial were selected based on the market results. The system that best fits most markets is a five-band scale: <200; 200–500; 500–1500; 1500–5000; >5000 kcells/ml (kcells = 1000's cells). However, it is possible to fine-tune these thresholds to the requirements of each specific market.

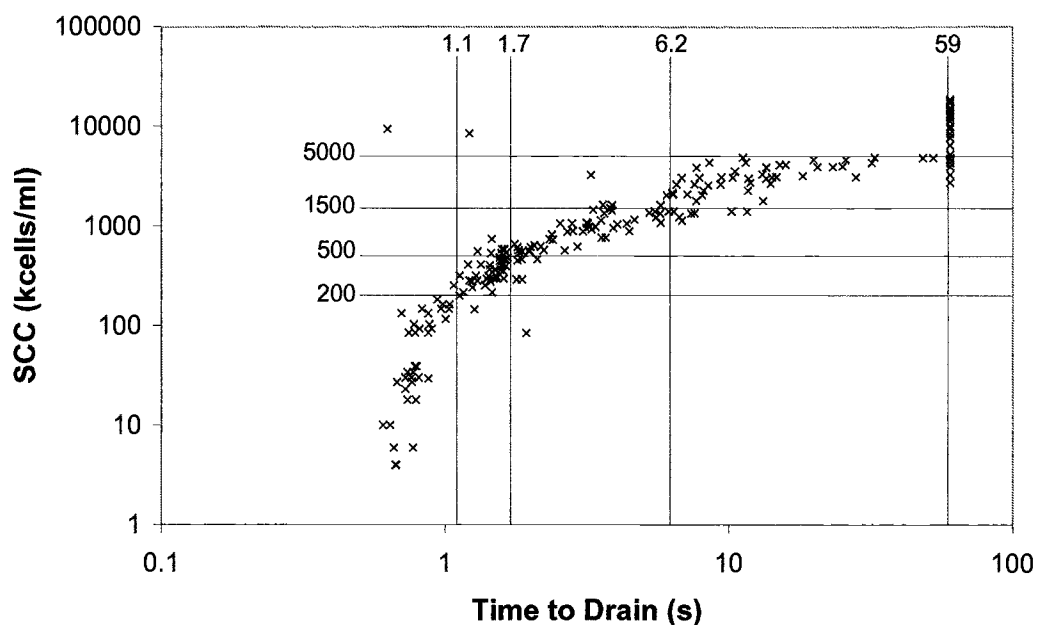
Table 1 and Figure 4 show the data leading to the thresholds chosen and the sensor performance in each band. The SCC sensor prototype described here has a level of performance adequate for use as a mastitis control tool and for managing cell concentration in the bulk milk tank.

Table 1 Summary of SCC sensor prototype performance: time-to-drain thresholds, the total number of milk samples (n), and the number of correct (n_{correct}) and proportion of correct (p) measurements from the calibration and on-line testing.

SCC band (kcells/ml)	Time-to-drain thresholds (s)	n (lab)	n _{correct} (p) (lab)	n (on-line)	n _{correct} (p) (on-line)
<200	<1.1	38	36 (95%)	64	62 (97%)
200 – 500	1.1 – 1.7	39	33 (85%)	2	2 (100%)
500 – 1500	1.7 – 6.2	59	45 (76%)	2	1 (50%)
1500 – 5000	6.2 – 59	60	43 (72%)	0	0 N/A
>5000	>59 ¹	42	40 (95%)	1	1 (100%)
Overall	N/A	238	197 (83%)	69	66 (96%)

¹The sensor time-out was set at 60 seconds

Figure 4 Calibration curve for SCC sensor prototype (lab testing only), showing the location of SCC band boundaries



Quarter Conductivity

Attempts to measure the electrical conductivity (EC) of milk as a means of detecting mastitis date back to the first half of the last century. Those early efforts (in common with several conductivity devices on the market today) failed, however, because of the normal biological variations in conductivity of milk that have nothing to do with mastitis. Historically, the value of EC as a mastitis detection tool has been disappointing, principally because the conductivity of milk is influenced by variations such as those listed below.

- From cow-to-cow within a herd, from herd-to-herd, from breed-to-breed
- Over the course of a lactation and in response to varying milking intervals
- Over the course of a milking in response to changing fat content of the milk
- In response to varying feed types and intake amounts
- In response to changes in milk temperature
- As a result of dirt, milk stone or milk fat build-up on the sensor electrodes

A quarter conductivity sensing system has been developed in an attempt to eliminate or control all the non-mastitic variables that can affect milk conductivity in order to focus solely on those factors that cause an increase in conductivity as a consequence of tissue damage within the cow's udder.

Examples of recent results

1. The results of a recent study in New Zealand's only automatic milking herd for the period August 1, 2002 to March 1, 2003 showed that:
 - All 8 quarters with clinical mastitis were correctly identified by quarter and date
 - 12 of the 13 quarters infected with a major mastitis pathogen were correctly identified by quarter and date
 - Only 7 of 26 quarters infected with minor pathogens appeared on the alarm list

If the presence of a major pathogen is defined as a "True positive", the Sensitivity was 92% (12 of 13 quarters) and Specificity was 95% (391 of 411 quarters).

This system is currently in use on robotic milking systems, where quarter milk is kept separate until it passes through the individual quarter conductivity sensors. It may be possible to apply this technology to conventional milking systems. We are currently working on methods of applying quarter separation to conventional claws.

REFERENCES

1. Claycomb R., Mein, G., Whyte, D. and Wilson, A. (2002) On-Line milk component sensing, *Proc 33rd ICAR Session*, Interlaken, Switzerland.
2. Davis, S.R., Farr, V.C., Prosser, C.G., Nicholas, G.D., Turner, S-A., Lee, J. and Hart, A.L. (2004) Milk L-lactate concentration is increased during mastitis. *J. Dairy Res.* **71**: 175-181.
3. Eckersall, P.D., Young, F.J., McComb, C., Hogarth, C.J., Safi, S., Weber, A., McDonald, T., Nolan, A.M. and Fitzpatrick, J.L. (2001) Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet. Rec.* **148**: 35-41.
4. Whiteside, W.H. (1939) Observations on a new test for the presence of mastitis in milk. *Can. Pub. Hlth. J.* **30**: 44.
5. Whyte, D., Walmsley, M., Liew, A., Claycomb, R. and Mein, G. (2004) Chemical and rheological aspects of gel formation in the California Mastitis Test. *J. Dairy Res.* (In press)