Sensors and Milk Quality – the Quest for the Perfect Alert

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Introduction

There are many aspects to milk quality. Mastitis is associated with two of the milk quality aspects that are used in most dairy producing countries: somatic cell count (SCC) and, if mastitis is clinical, visibly abnormal milk (in the remaining part of this article referred to as abnormal milk). In most dairy systems it is assumed that the farmer, informed by the official organizations in his country, has the responsibility to deliver milk of sufficient quality. In order to deliver milk with a low SCC, attention should be given to an adequate detection and prevention of mastitis. Efficient detection of clinical mastitis is therefore important. A well-established method to detect clinical mastitis is to strip before milking and check the foremilk for abnormalities. Discarding of abnormal milk is part of the EU Milk Hygiene Directive (EC/92/46). Milk from diseased cows or milk that is visually abnormal should not be delivered. Discarding of abnormal milk is also mandatory in the USA, according to the Grade "A" Pasteurized Milk Ordinance. Checking of foremilk is thus important to detect clinical mastitis, depending on the regulations of a country, to meet regulations.

After development of individual animal identification, applications have been sought for. Individual feeding, individual milking and automated detection of events of interest for the farmer are all applications following the development of individual animal identification. Because of the costs of the mastitis (Halasa et al., 2007; Huijps et al., 2008), this disease has been the first focus of sensor developments in the dairy sector. From the mid-eighties, work has been carried out in order to automate the detection of mastitis by means of sensors. During milking, abnormal milk was detected using visual observations (Rasmussen, 2005; Rasmussen and Bjerring, 2005). When detection of clinical mastitis is carried out automatically, the task of the milker becomes easier and the capacity of milking parlous can be increased. Although sensors for detection of mastitis became commercially available in the beginning of the nineties, they never were applied in a large scale. Because of the fact that with automatic milking no milker is present at the time of milking, the need for sensors to detect clinical mastitis and abnormal milk was high when automatic milking systems were commercially introduced. Moreover, because of the number of milking clusters in an automatic milking system is much lower than in a comparable milking parlor, the costs of application of sensors is also lower in an automatic milking system. Therefore, interest in the application of sensors to detect mastitis and abnormal milk has been gaining considerably in the past years.

Until now, performance of milk quality sensors has been adequate, but not great. Because sensors are more widely applied, especially because of automatic milking, there is a strong need for

improvement of performance. There are two major routes through which this can be done: improvement of sensors and improvement of detection models that translate the sensor data in information for the herdsman. This paper describes the current status of sensors to detect abnormal milk, subclinical mastitis and clinical mastitis. To evaluate the performance of a system, the goal of usage determines the needed performance. Therefore, this paper first starts with a section on performance demands, followed by a section on different types of sensors, different approaches of algorithms and will finish with an overview of performance of systems.

Demands for Automatic Detection of Mastitis and Abnormal Milk

Evaluation of sensors

Sensors for detection of mastitis and/or abnormal milk can be seen as diagnostic tests, which can be characterized by epidemiological parameters. When evaluating a sensor, this is done in an experiment where the alerts given by the sensor are compared with the occurrence of an event in reality (gold standard). The outcomes of such an experiment can be classified as follows:

- Observations where the event occurs with an alert (*TruePosCount*)
- Observations where the event occurs without an alert (*FalseNegCount*)
- Observations where the event does not occur with an alert (*FalsePosCount*)
- Observations where the event does not occur without an alert (*TrueNegCount*)

Using these basic classifications, the performance of a sensor can be evaluated as follows. The two most important parameters are sensitivity and specificity:

*Sensitivity (%) = 100 * TruePosCount / (TruePosCount + FalseNegCount*
*Specificity (%) = 100 * TrueNegCount / (FalePosCount + TrueNegCount)*

The sensitivity refers to the probability that the event of interest (e.g., a cow with clinical mastitis) will be classified as such (positive test result; alert). The specificity refers to the probability that when the event of interest does not occur (e.g., a without clinical mastitis) will be classified as normal (negative test result; no alert). Sensitivity and specificity are interdependent. If the threshold of a test is increased, the number of positive outcomes and thus the sensitivity will decrease. On the other hand, the specificity will increase. Therefore, thresholds have to be set in such a way that the performance of a sensor in terms of sensitivity and specificity is optimized.

The sensitivity and specificity of a test are independent of the occurrence of the events (prevalence). For a practical evaluation of sensors, the prevalence of the event of interest is important. The prevalence of clinical mastitis, subclinical mastitis and abnormal milk is very low. Prevalence of clinical mastitis and abnormal milk is approximately 0,04 % (4 cases per 10,000 milkings). The prevalence of subclinical mastitis is, depending on the definition of subclinical mastitis, approximately 100-5000 cases per 10,000 milkings. This low prevalence, especially for clinical mastitis, will have effects on the interpretation of sensor data. The farmer does not see the gold standard, but sees alerts. To evaluate sensors to detect mastitis or abnormal milk from a farmer's point of view, the following two definitions are proposed (Sherlock et al., 2008):

Success Rate = TruePosCount / (TruePosCount + FalePosCount
*Fale Alert Rate = 1,000 * FalsePosCount / totalCowMilkings*

Note that we have deliberately avoided the commonly employed terms true positive, false positive, true negative and false negative because of inconsistency in their usage. For instance, consider the following three definitions of a true positive as: 'a case of mastitis where one or more alerts are given' (deMol et al. 1997); 'an alert during a mastitis period' (deMol and Woldt, 2001); and 'an alert on the day of observation' (Cavero et al. 2006). Each is capable of generating a different true positive list from the same basic data. Success rate could be a more useful statistic, giving a more direct measure of the proportion of alerts that are likely to be correct. Success rate is a synonym for the positive predictive value. A downside of success rate is that it is not an 'absolute' statistic. Thus, the success rate will vary with the prevalence of the condition being monitored. This downside can be avoided by calculating the total number of false alerts over a given number of cow-milkings, e.g., the total number of false alerts per 1,000 cow-milkings. This expression of the 'false alert rate' would be a simple, practical and comprehensible measure to which farmers will readily relate. The false alert rate is essentially 10 * (100% - specificity) per 1,000 cow-milkings. This approximation should always be close enough for practical purposes since in normal situations, the prevalence of mastitis or abnormal milk is very low relative to the total number of cow milkings.

A basic evaluation method is to compare alerts with events at the same moment. For instance, for 1,000 milkings visual evaluation of normality of milk is carried out. These data are used as the gold standard. An event is defined as the occurrence of abnormal milk. When, during the same milking the sensor is used and alerts are generated, the counts of events in relation to alerts can be evaluated as above (e.g., Mollenhorst et al., 2010; Nielen et al., 1995a). However, when the timing of observations of events and alerts does not occur at the same moment, time windows have to be used to combine those observations (e.g. Kamphuis et al., 2010; de Mol et al.). Especially when the time between observations is variable, the use of a time window becomes more complex (Sherlock et al., 2008). Time windows can also be used in the interpretation of timing of alerts. If it is no problem when an alert is given up to fourteen days after the onset of an event, an alert will regarded as true positive, when it is given within fourteen days of the onset of the event. This knowledge can be used calculating the TruePosCount and TrueNegCount. In general, performance of sensors will improve when larger time windows are used. When the used time window during evaluation does not match practice, the actual use of sensors might lead to disappointment by the farmer. Therefore, time windows should be well considered.

In the evaluation of sensors, it is very important that the event of interest is clearly defined; especially because the demands for a test might differ for the event of interest. For instance, detection of (visual) abnormal milk is done for different purposes than detection of clinical mastitis or subclinical mastitis. In the following sections these events will be described in the light of demand for performance of sensors.

Clinical mastitis

The primary goal for on-line detection of clinical mastitis is to be able to cure the diseased cow. After an alert signal, the herdsman will check the cow first to confirm the mastitis before deciding on treatment. The advantage of using a sensor system to detect clinical mastitis is the management by exception principle. Only those cows requiring attention will get it. Because a mastitis case will be confirmed first, a little higher false alert rate will not be a problem. The only costs that are made are the check made by the herdsman. Alerts can be given directly in the milking parlor, decreasing

the time that is needed to milk one cow. Currently, sensors to detect clinical mastitis are mostly applied in automatic milking systems. In these systems, alerts are placed on a list. The herdsman checks this list regularly and has to checks the cows on it. Costs involved with checking are higher, because it requires more (annoying) labor to find the cow, fetch her and check her somewhere in the barn. For use with an automatic milking system, therefore, the false alert rate should be lower. It is important that as many cows with clinical mastitis as possible (preferably all) will be identified, requiring a high sensitivity. From a welfare point of view, at least cows with severe clinical mastitis (grave systemic and local symptoms) must be detected. Time windows for clinical mastitis should be short. A cow with clinical mastitis should be treated soon after the onset of clinical signs. When a cow receives an alert when there are no clinical signs yet, the farmer will check the cow and determine that it was a false alert and do nothing, while in fact the cow would become clinical soon. In the next section, this will be discussed further.

Using research results from a large European project (Rasmussen and Bjerring, 2003), the latest International Standard (ISO 20966, 2007) includes an Annex, attempting to deal with methods of detecting abnormal milk and interpretation of test results. This annex describes a minimum sensitivity of 80 %, combined with a specificity larger than 99 % (~false alert rate smaller than 10 per 1,000 milkings).

Subclinical mastitis

Events are only interesting when detection offers opportunity for improvement. This is difficult to define in the case of subclinical mastitis. First of all, the definition of subclinical mastitis is difficult. There might be three possibilities for which detection of subclinical mastitis is interesting, and the appropriate definition of subclinical mastitis depends on these. Mastitis is caused by infection. Before a cow with an intramammary infection becomes clinical, there is a subclinical phase, where in presence of a pathogen, the somatic cell count is already elevated. In a controlled experiment it has been shown that the efficacy of treatment improves when cows with an intramammary infection are treated before occurrence of signs of clinical mastitis (pre-clinical mastitis). When treating cows based on alerts of a sensor system before clinical signs appear, the false alert rate should be very low, because for each false alert, a cow is treated with antibiotics. The sensitivity does not necessarily have to be high, because common practice at the moment is to wait with treatment until a cow becomes clinical. Time windows can be longer than for clinical mastitis.

Besides the treatment of cows with subclinical intramammary infection shortly before onset of clinical signs, cows with chronic subclinical mastitis might also be treated. Until now, research did not show much economic benefit of treatment of subclinical mastitis (Swinkels et al., 2006; Steeneveld et al., 2007). When modeling with a dynamic model, the reduction of transmission of infections within a herd gave clear economic benefits of treatment of subclinical mastitis (Van den Borne et al., 2010). Although the use of somatic cell counts, as measured in a dairy herd information program are sufficient to detect cows with chronic subclinical mastitis, sensors can be used for that task. The false alert rate should be low, but there are no high requirements for sensitivity or time windows.

Finally, the milk payments depend on the bulk milk somatic cell count. If that exceeds a certain limit, a bonus is missed or a penalty is given. If the bulk milk somatic cell count of a farm is close to a payment limit, it might be beneficial to not deliver milk of certain cows with a high somatic cell count. Sensors may assist in this. Again, false alert rates should be low. The sensitivity

is not very important because only milk of a limited number of cows has to be excluded from delivery. Short time windows are more important because the milk that is excluded from delivery should indeed by milk with a high somatic cell count.

Abnormal milk

Almost all abnormal milk is a result of clinical mastitis. However, abnormal milk might also be caused by blood. While the goal for detection clinical mastitis is to be able to cure a diseased cow, the goal of detection of abnormal milk is to discard this milk, as is required by regulations in most milk producing countries. If the task of discarding abnormal milk has to be carried out automatically as is the case with an automatic milking system, it is not sufficient to use alert lists. An alert must be followed directly by automatic separation of the milk. The management information system must detect as much milkings with abnormal milk as possible (preferably all), requiring a high sensitivity. Since each milking which is unnecessarily separated costs money, the false alert rate should be very low. Time windows should be small.

The costs associated with automated discarding of abnormal milk under varying performance of sensor systems have been evaluated (Pietersma and Hogeveen, 2004). The results of this research suggested that the false alert rate of detection systems for automatic separation of abnormal milk should be very low, perhaps lower than 5 false alerts per 1,000 milkings, to avoid substantial economic losses due to incorrectly discarding normal milk.

Sensors to Detect Mastitis and Abnormal Milk

Because of the physiological changes in the udder, intramammary infections lead to major alterations in the composition of milk (Kitchen, 1981). Clinical mastitis is per definition an intramammary infection where visible changes in the appearance of the milk, the udder or both occur as an effect of the inflammation process. Amongst others, a rapid influx of polymorphonuclear leukocytes leads to an increase of the somatic cell count (SCC). This SCC is the basis of many milk quality programs worldwide. Besides the influx of polymorphonuclear leukocytes, there are other compositional changes in milk. Sensors have been developed that can detect some of these changes on-line. Recently, two reviews have been published on sensors and udder health (Brandt et al., 2010; Viguier et al., 2009) This chapter presents a summary of the most important sensors, currently available in practice.

Electrical conductivity

Electrical conductivity (EC) is a measure of the resistance of a particular material to an electric current. In milk, ions present are the main component conducting electricity. Active and passive transport systems in the secretory cells of the mammary gland keep the sodium-potassium ratio in the milk approximately 1:3, whereas it is 30:1 in extracellular fluid or blood. The chloride concentration in milk is much lower than in blood. The mammary ducts are impermeable to ions (Figure 1A). Mastitis leads to a change in blood capillary permeability, destruction of tight junctions and the destruction of the active ion-pumping systems. As a result the ion concentrations in milk change. Since milk is iso-osmotic with blood, the secretory cells of the mammary gland will stabilize the osmotic pressure leading to a change in EC (Figure 1B).

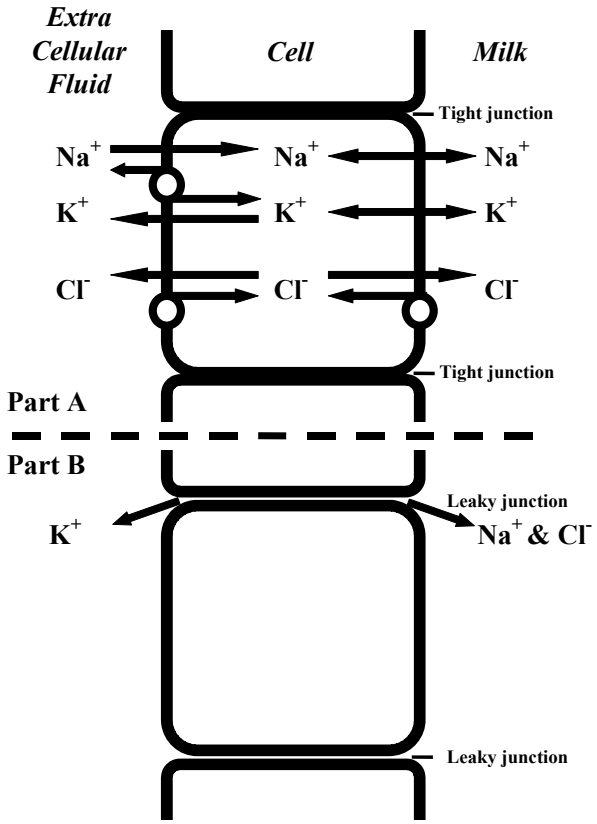

Figure 1. Schematic overview of the pathways of ion transport in the mammary secretory epithelium (based on Linzell and Peaker, 1971). Part A is the situation in a normal functioning mammary gland. Part B represents some of the changes caused, amongst others, by mastitis.

Because the principle of measuring EC is relatively simple, sensors for measuring EC are commercially available for a number of years. Basically there are two types of systems available: 1) systems that measure the conductivity of the whole milk, located for instance in the electronic milk meter and 2) systems measuring the conductivity per udder quarter, located in the claw of the milking cluster (traditional milking systems) or in the long milk tube (automatic milking systems). Since mastitis is an event which occurs on udder quarter, EC measurements on quarter level give the possibility to compare udder quarters, thus increasing the test characteristics.

L-lactate dehydrogenase

L-lactate dehydrogenase (LDH) is the result of one of the enzymatic reactions following mastitis. It is part of the glycolytic pathway, found in the cytoplasm of all cells and tissues in the body. LDH is a responsive indicator of mastitis as a result of the animal's immune response against infection and changes in cellular membrane chemistry. . A bio-sensor, using dry-stick technology is commercially available commercially and has been evaluated.

Color

A direct measure of the physical characteristics of abnormal milk (mostly due to clinical mastitis) will most likely offer better detection results than a measurement of an indirect indicator of mastitis or abnormal milk. One of the visible aspects of milk is its colour. Recently a sensor for on-line colour measurement is on the market.
The principle of the sensor is based on the reflection of light generated by a LED. The whiter the milk, the more light is reflected. Three different wavelengths of light are measured by the sensor: red, green and blue.

In a first study under laboratory circumstances, using homogenised quarter milk samples from 8 cows with clinical mastitis the potential to detect mastitis from colour measurements was estimated. The milk samples of the suspected quarters of all 8 cows with clinical mastitis showed lower color values than homogenized milk.

Color sensors are commercially available. In a detailed study on the predictive potential of EC and colour measurements, it became clear that most information to distinguish udder quarters with abnormal milk and clinical mastitis from other udder quarters could be found in EC measurements. The potential of colour measurements did add but not very much. This means that colour sensors should always be used in combination with other sensors.

Somatic cell count

The best-known and most widely applied parameter related to mastitis and used for detection, is the somatic cell count (SCC). Rapid reliable measurement of SCC is carried out routinely in laboratories, and can be used to monitor udder health. Therefore, SCC is used as an important tool for the control of (subclinical) mastitis. Near infrared (NIR) has shown to be able to measure, amongst others, SCC in raw milk (e.g., Tsenkova et al., 1999). A commercial available NIR analyzer has been described (Katz et al., 2007).

Sensors that measure SCC on-line, based on the principles of CMT are commercially available on automatic milking systems. One of these sensors utilizes the gel-formation process of the Californian mastitis test (Figure 2). The potential value of this sensor has been studied at the cow level in combination with quarter-based EC. With thresholds set in such a way that the sensitivity of the test was 80 %, on-line SCC measurement gave similar results as EC (using standard algorithms). The test based on on-line SCC measurement gave a probability of 13 % that a positive sensor outcome was a case of clinical mastitis. When combining EC and SCC measurements, the probability that a positive sensor outcome was a case of clinical mastitis increased to 33 %. These results suggested that estimating SCC from a composite cow milking contributes to an automatic sensing system for the detection of CM by reducing the number of false positive attentions while keeping the sensitivity of detection at a reasonable level. Recent, yet unpublished, data show that measuring SCC on quarter level gives better detection performance than measuring SCC on cow level.

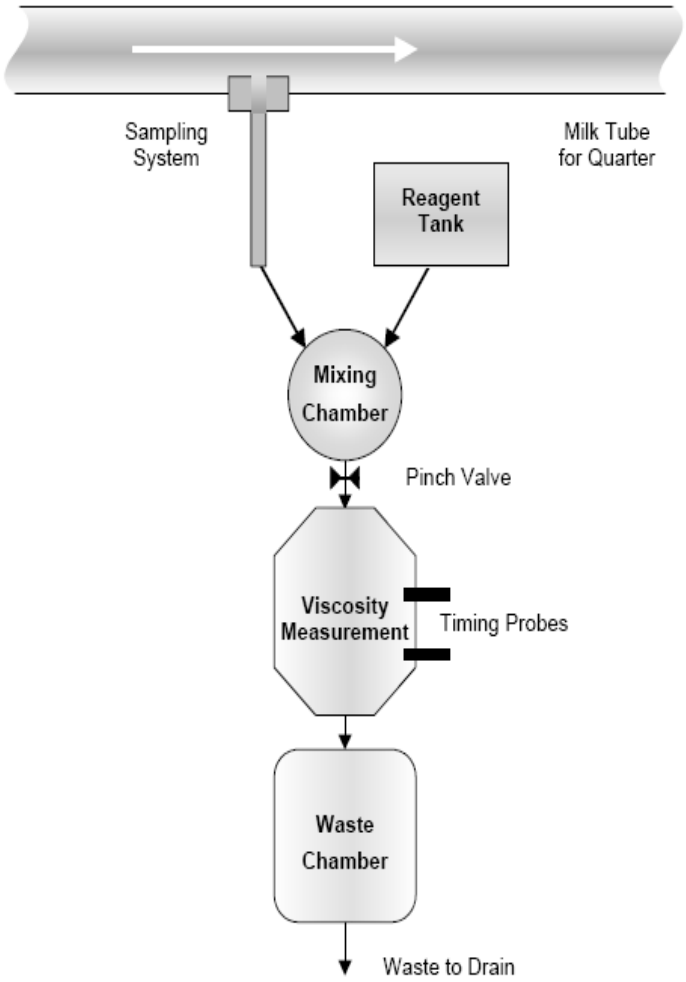

Figure 2. Representation of on-line SCC sensor based on gel formation as utilized by the Californian mastitis test.

Algorithms and Performance

Sensors, how advanced they might be, deliver data. Many on-line sensors deliver a large amount of data. There are many measurements per milking, sometimes per udder quarter and during many milkings. These data in itself are not informative. These data should be processed to generate information.

An example of information is the probability that this cow has mastitis during this milking (alerts). Many algorithms have been proposed, developed and described to perform this task. A very straightforward example of an algorithm is the use of a threshold: if the measurement is above that threshold, an alert is generated. Because of the low prevalence of the event that has to be detected (clinical mastitis, abnormal milk or subclinical mastitis), there are high demands on the processing of sensor data. A good algorithm is essential to optimize the on-line sensor data in an interpretable value. Algorithms can make a huge difference in the performance of a sensor system. Described algorithms include the use of thresholds (e.g., Mollenhorst et al., 2010), moving averages (e.g.,

Maatje et al., 1992), neural networks (e.g., Nielen et al., 1995a), multivariate regression models (e.g., De Mol et al., 1997; Nielen et al., 1995b), time series models models such as Kalman filters (e.g., De Mol et al., 1999; Friggens et al., 2007), fuzzy logic (e.g. Cavero et al., 2006; De Mol and Woldt, 2001) and datamining (e.g., Kamphuis et al., 2010). The information should finally be processed into action. Many times this latter step is carried out by the herdsman, although there are possibilities to support this with automation (automated decision support).

Table 1. Overview of performance, including sensitivity (SE), specificity (SP) and used time window of detection models using measurement of electrical conductivity (EC) as main sensor.

Paper	SE	SP	Time window	Definition of event	Sensors
Cavero et al., 2006	81	94	5d	Treated cases of mastitis	EC, milk prod, milk flow
De Mol & Ouweltjes, 2001	100	96	7d	Observation of clinical mastitis	EC, milk yield
De Mol & Woldt, 2001	100[a]	99.75	7d	Observed Clinical mastitis	EC
De Mol et al., 1997	90[b]	98.3[c]	17d	Clinical mastitis	EC, Milk yield, temp
De Mol et al., 1997	76[b]	98.3[c]	28d	Sublinical mastitis	EC, Milk yield, temp
De Mol et al., 2001	71[b,d]	97.9[c]	4d	Clinical mastitis	EC, Milk yield, temp
Maatje et al., 1992	100	?	14d	Clinical mastitis	EC, temp, milk yield
Maatje et al., 1997	90[b]	98.2[c]	14d	Clinical mastitis	EC, temp, milk yield
Mottram et al., 2007	56	82	-	Clinical mastitis	EC
Nielen et al., 1995	77	69	1d	Clinical mastitis	EC, temp, milk yield
Nielen et al., 1995f	84	97	0	Clinical mastitis	EC, temp, milk yield
Norberg et al., 2004	43	93	0	Clinical mastitis	EC
Claycomb et al., 2009	83	98[e]	4d/2d	Clinical mastitis	EC
Sheldrake & Hoare, 1981	49	79	0	Mastitis, bacterio-logical culturing	EC

[a] fuzzy logic was used to classify alerts generated by detection model previously developed (De Mol and Ouweltjes, 2000) in order to decrease FP alerts, not to increase SE of the detection model
[b] calculated for a mastitis case
[c] calculated for a mastitis-free milking using only cows that never had mastitis
[d] average SE, based on two alert lists (one for clinical mastitis, one for illness in general)
[e] approximation using formula: false alert rate ≈ 10 * (100 – specificity)

Table 2. Overview of performance, including sensitivity (SE), specificity (SP) and used time window of detection models using measurement other sensors than only electrical conductivity (EC) as main sensor.

Paper	SE	SP	Time window	Definition of event	Sensors
Kamphuis et al., 2008	80	92[a]	3d	Treated cases of masitits	EC, in-line somatic cell count
Kamphuis et al., 2010	32	98.7	<1d	Clinical mastitis	EC, color, milk prod
Kramer et al., 2009	75	92.1	5d	Treated cases of mastitis	Milk yield, dry matter intake, water intake, activity, previous diseases
Friggens et al., 2007[b]	92.8	97.9	15 days	Treated cases of mastitis	L-lactate dehydrogenase

[a] approximation using formula: false alert rate ≈ 10 * (100 – specificity)
[b] including only a highly selected set with only clearly diseased and clearly healthy cases.

Because mastitis is associated with many changes in the cow and milk, a combination of more than one sensor has been proven to be useful. The most used sensor for EC, is many times combined with measures of milk yield and milk temperature (Table 1). Sensitivity varies from 43% tot 100 %, while the specificity varies from 69 % tot 99.75 %. However, used time windows do also vary greatly from 0 days (measurements during the same milking) to 28 days. Although in some studies a sensitivity of 100 % was found when using EC to detect clinical mastitis, test results in studies more closely related to practice are not very good. Especially the sensitivity is low. Table 2 gives an overview of more recent studies that use newly developed sensor such as a sensor for color, somatic cell count or LDH. A final paper (Kramer et al., 2009) is included in Table 2 that uses many measurements carried out outside the milk. Also in these studies there is a large variation in found sensitivities, specificities and time windows.

The variation in study results is difficult to explain. There is a relation between sensitivity and specificity. By varying the classification threshold, the sensitivity might be changed. This change is negatively correlated with a change in specificity. Another interesting observation is the relation between used time window of evaluation and performance of the system. With a longer time window, the performance increases (Figure 3).

A combination of data from different sensors has shown to improve the detection performance (e.g., Kamphuis et al., 2008b; Mollenhorst et al., 2010). This combination needs to be accompanied by improved and more complex detection algorithms. A final method that is proposed to improve the detection performance of sensors, is the combination of sensor output with other, non-sensor information of the cows, such as lactation stage, mastitis history (Chagunda et al., 2006; Steeneveld et al., 2008). It is applied in one described system (Chagunda et al., 2006), but from that publication it was not clear what the addition of the non-sensor information added to the detection performance. Very recent data showed that, although cow information does predict the risk of clinical mastitis and thus the prior probability of clinical mastitis, these additional data did not add

much to the detection performance of sensor systems (Steeneveld et al., 2010). Therefore, not too much must be expected from this combination of sensor data and other cow information.

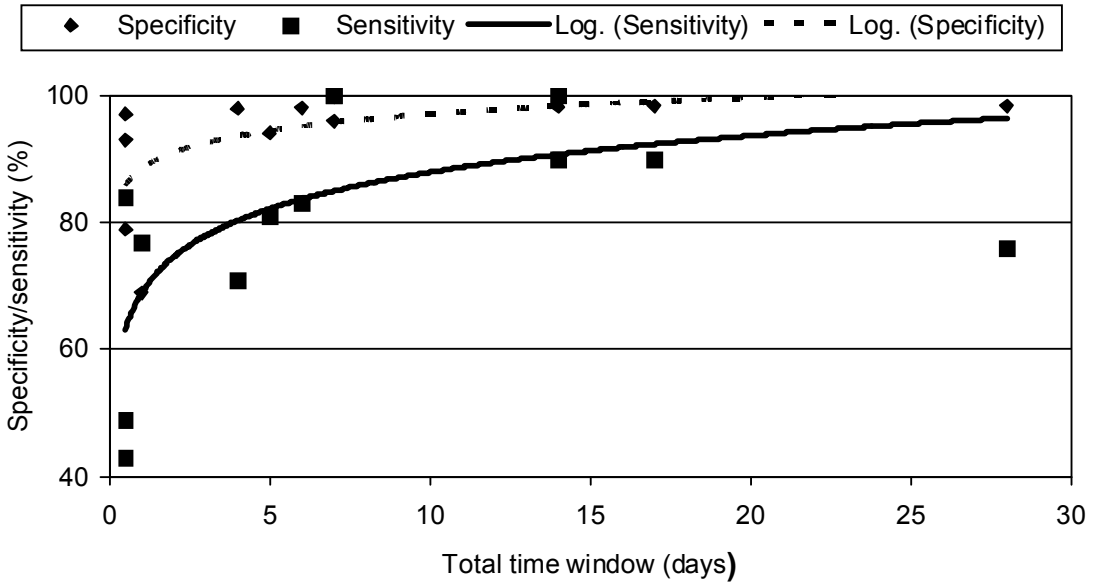

Figure 3. Sensitivities and specificities of various studies, plotted against the used time windows.

Concluding Remarks

The use of sensors to improve milk quality gains much attention lately. This is largely because of the demand of good performing sensors to be used by automatic milking systems. The mostly studied and applied sensor measures EC. Besides the development of automatic milking systems, there are new sensor developments, for instance the use of NIR, measurement of SCC and LDH that makes interesting future improvement possible. When evaluation a sensor system, it is very important to define the event that needs to be detected. Moreover it is important to consider an appropriate time window. For the most needed detection systems, to detect clinical mastitis, a short time window should be used.

For future applications, the definition of mastitis might be reconsidered. Currently there is still a distinction between clinical and subclinical mastitis. With renewed interest and found efficiency of treating subclinical mastitis cases, it might be interesting to get away from a binary mastitis variable and to go to a continuous mastitis variable. A first step towards such a system of thinking has recently been made with the introduction of "Degree of infection" (Højsgaard and Friggens, 2010). Currently we are at the beginning of a series of new developments. The quest for the perfect milk quality is only beginning.

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