# Mastitis diagnostics by near infrared spectra of cow's milk, blood and urine using soft independent modelling of class analogy classification

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## Introduction

Biological fluids, such as milk, blood and urine, contain information specifically related to metabolic and health status of ruminant animals. Some changes in their composition can be attributed to the disease response in the animals. Finding these changes soon after they have occurred could lead to an early diagnosis followed by more effective treatment or even disease prevention.

Mastitis is a disease in a cow's mammary gland, initiated when bacteria enter the gland and establish an infection or inflammation.[1] It causes major problem for the global dairy industry and substantial economic losses from decreasing milk production and reduced milk quality. When mastitis occurs, lactose in milk decreases and lactose in urine increases.[2,3] Alteration in the blood–milk barrier leads to an influx of blood proteins in milk and milk proteins in blood, such as α-lactoalbumin and casein, and to changes in ionic concentration in milk.[4–6] Mastitis is accompanied by an influx of white cells from the blood stream into the milk, altered secretary function and changes in the volume and composition of secretion. Somatic cell count (SCC) has been accepted as the international standard measurement of dairy milk quality and mastitis diagnosis.[7] In previous studies, near infrared (NIR) has been successfully applied for noninvasive mastitis diagnosis performed by qualitative raw milk spectral analysis[8,9] when an expert's diagnosis has been used as a reference. NIR diagnosis has been based on compositional changes in milk caused by mastitis and their respective spectral changes.

In this study, for the first time, it was found that mastitis diagnosis could be performed with equal success when NIR spectra of any biological fluid, such as milk, blood or urine, were classified using SCC in milk as a disease threshold.

## Materials and methods

### Samples

A total of 112 bulk milk, urine and blood samples from four Holstein cows were analysed. The samples were collected for 28 days, consecutively, starting from 7$^{th}$ day after calving. The milk samples were collected from morning milking. The urine samples were collected before morning milking and stored at –35°C until spectral analysis. The blood samples were collected before morning milking using a catheter inserted into the carotid vein. Heparin was added to blood samples to prevent coagulation. The average BW of the cows was 552 kg.



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Each milk sample was divided into two subsamples. One was subjected to spectral analysis and the other was analysed for SCC by fluoro-opto-electronic method using a Fossomatic 400 (Foss-Electric A/C, Hillerød, Denmark). Somatic cell count standards were used to calibrate the Foss instrument throughout the study. The repeatability coefficients of variation of this method are 4 to 5% for the region between 400,000 and 500,000 cells $mL^{-1}$ and 5 to 10% for the regions between 100,000 and 200,000 cells $mL^{-1}$ and over 500,000 cells $mL^{-1}$ (IDF Standard 148A, 1995).[7] The SCC content in milk was used as the indicator for mastitis. One cow was mastitic during the entire experimental period—the measured SCC varied from 204,000 to 11,876 ,000 cells $mL^{-1}$. Three of the cows had mastitis and healthy periods—SCC was between 80,000 and 437,000 cells $mL^{-1}$.

SCC values of milk samples were used as the quantitative parameter for respective urine and blood samples collected from the same cow, at the same time.

### NIR spectra

Near infrared transflectance (T) spectra of blood and milk samples were obtained using an InfraAlyzer 500 spectrophotometer, (Bran+Luebbe, Nordestedt, Germany), in terms of optical density log (1/*T*) in a wavelength range from 1100 to 2500 nm. A flow cell, with a pathlength of 0.2 mm, connected with an automated liquid sampling system taking, alternatively, milk samples and cleaning solution, was used. Before the spectral analysis, each sample was warmed up to 40°C in a water bath with a temperature control of ± 0.1°C. During the analysis, the same temperature was controlled through the use of an integrated water-jacketed holder of the flow cell connected with the water bath.

Near infrared spectra of urine samples were obtained using an NIRSystem 6500 spectrophotometer (Foss NIRSystems, Silver Spring, MD, USA), using quartz cuvettes with 1 mm sample thickness, in the spectral region from 1100 nm to 2500 nm.

### NIR analysis

A commercial program, Pirouette Version 2.6 (Infometrics, Inc., Woodinville, WA, USA), was used for qualitative analysis, i.e. classification of samples. The spectral region from 1100 to 2500 nm was used in calculations for all data sets. Classification of milk samples in class "healthy" or class "mastitic" was done using soft independent modelling of class analogy (SIMCA). The examined methods for data pretreatment included smoothing of the spectral data, first and second derivative transformation of log (1/*T*) data, based on the Savitzki–Golay second-order polynomial filter.[10] All samples were divided into a calibration set (two thirds of the samples) and a test set (one third of the samples). A class variable was assigned for each sample as follows: healthy (class 1)—with SCC lower than 200,000 cells $mL^{-1}$ and mastitic (class 2)—with SCC higher than 200,000 cells $mL^{-1}$. The calibration set of samples consisted of 75 samples—30 samples from healthy cows and 45 samples from mastitic cows. The test set consisted of 37 samples—17 samples from healthy cows and 20 samples from mastitic cows. SIMCA developed models for each class based on factor analysis, i.e. principal components that describe the variations of the spectral data. Loadings of the principal components for each model were compared.

## Results and discussion

SIMCA classification of milk, urine and blood spectra (Figure 1), respectively, based on somatic cell count (SCC) in milk as a threshold for mastitis diagnosis, is presented in Table 1. For the calibration set of samples, SIMCA models (model for samples from healthy cows: class 1 and model for samples from mastitic cows: class 2), correctly classified from 97.33 to 98.67% of milk samples, 97.33 to 98.67% of urine samples and 94.67 to 96.00 % of blood samples. The best results for all data sets were obtained when first derivative spectral data pretreatment was used. Incorrectly classified samples were false negative, false positive or non-classified. Most of them had an SCC close to the defined threshold

**Table 1. Results for SIMCA classification based on milk, urine and blood spectra (class 1: samples from cows with milk SCC < 2000,000 cell mL$^{-1}$, class 2: samples from cows with milk with SCC > 200,000 cell mL$^{-1}$.**

| Spectra | Spectral data pretreatment | Calibration set $n$ = 75 | | | | Test set $n$ = 37 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | correct classification | | incorrect classification | | correct classification | | incorrect classification | |
| | | $n$ | % | $n$ | kind | $n$ | % | $n$ | kind |
| Milk | Smooth | 73 | 97.33 | 2 | 1 false negative<br>1 false positive | 26 | 70.27 | 11 | 2 false negative<br>6 false positive<br>3 non classified |
| | First derivative | 73 | 97.33 | 2 | 1 false negative<br>1 false positive | 32 | 86.49 | 5 | 1 false negative<br>4 false positive |
| | Second derivative | 74 | 98.67 | 1 | 1 false negative | 25 | 67.57 | 12 | 7 false negative<br>5 false positive |
| Urine | Smooth | 74 | 98.67 | 1 | 1 false negative | 27 | 72.97 | 10 | 3 false negative<br>4 false positive<br>3 non classified |
| | First derivative | 73 | 97.33 | 2 | 2 false positive | 32 | 86.49 | 5 | 4 false positive<br>1 non-classified |
| | Second derivative | 74 | 98.67 | 1 | 1 false negative | 31 | 83.78 | 6 | 1 false negative<br>4 false positive<br>1 nonclassified |
| Blood | Smooth | 71 | 98.67 | 4 | 2 false negative<br>2 false positive | 30 | 81.08 | 7 | 4 false negative<br>3 false positive |
| | First derivative | 72 | 96.00 | 3 | 2 false negative<br>1 false positive | 33 | 89.19 | 4 | 1 false negative<br>3 false positive |
| | Second derivative | 72 | 96.00 | 3 | 1 false negative<br>2 false positive | 29 | 78.38 | 8 | 2 false negative<br>6 false positive |

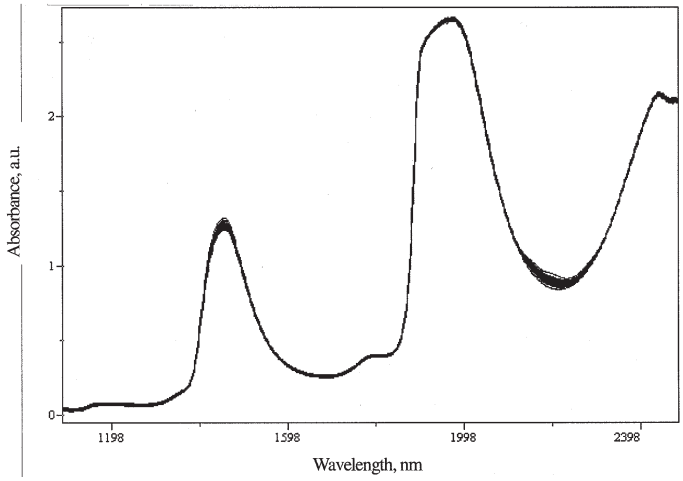


**Figure 1. NIR spectra of urine, 1 mm cell.**

of 200,000 cell $mL^{-1}$. There were more false positive samples than false negative ones. This result could be explained by the fact that SCC elevation was a result of the cow's defense mechanism against mastitis and, as such, post infection event was preceded by compositional changes in the biological fluids. These changes were reflected in the cow's milk, urine and blood NIR spectra, respectively. SIMCA models detected these changes earlier and classified the samples as samples from mastitic cows, although their SCC was still low.

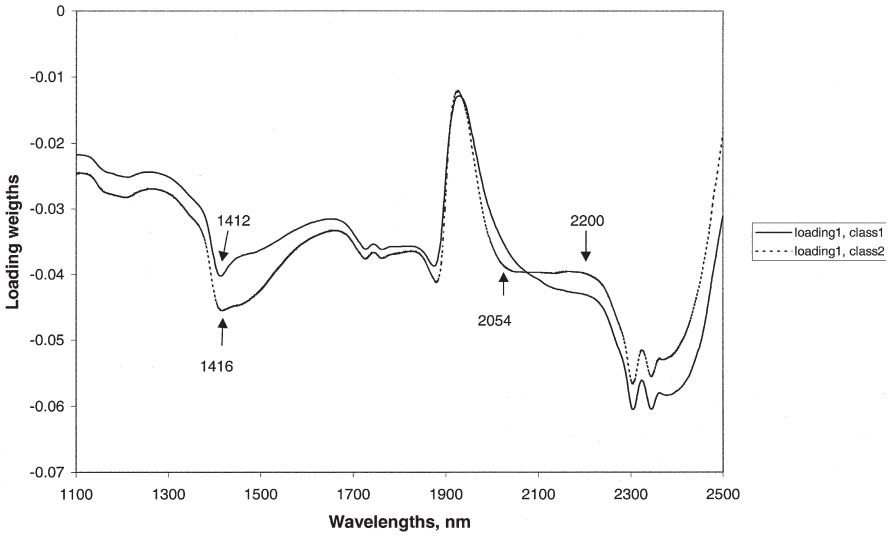


**Figure 2. NIR milk spectra: first principal component loading plots of SIMCA models for healthy cows (class 1) and mastitic cows (class 2).—— class 1, - - - - -class 2.**

SIMCA models were based on principal components (PC). Their loadings indicated variables (wavelengths) that had the highest contribution in the respective model and loading. Loadings of first PC components included in the SIMCA models were investigated. The first loading describes most of the variations in the related spectral data set. Plots of the first loadings for healthy and mastitic models, based on milk spectra, are presented in Figure 2. The first PC component described 96.88% of the total variations in healthy milk spectra (class 1) and 93.17% of the total variations in mastitic milk spectra (class 2). The respective values of total spectral variation, described by the first PC in the models for class 1 and class 2, were 87.71% and 88.57% for blood samples and 57.47% and 60.15% for urine samples, respectively. Differences in the spectral patterns of the first loadings were analysed in terms of loading weights and shape of the loading at characteristic wavelengths. Significant differences were found in the range between 1412 and 1450 nm for all of the tested biological fluids. For milk spectra, differences in weight and shape of the loadings were observed while for urine and blood spectral shifts in the position of maximum or minimum of the loadings were found, too. NIR absorption in this wavelength range is connected with the O–H absorption of water.[11] Characteristic differences in the loadings of both models, for the investigated fluids, were found in another water absorbance band at around 1910 and 1940 nm, too. It is known that mastitis markedly changes the ionic concentration in milk and blood.[6] Sodium and chloride contents in the milk increase because of the passage from the blood into the milk. Potassium declines because of the passage out of the alveolar lumen between damaged epithelial cells. The changes in blood cause respective changes in urine. Altered ionic concentration in milk, blood and urine exerted influence on water structure due to ion–OH interactions. We speculated that these changes were origininally from the observed differences in the NIR spectra of biological fluids[12] that were used to build up each SIMCA model for classification. PC loadings elucidated significant spectral differences from 2040 to 2200 nm for milk spectra, from 2140 to 2300 nm for blood spectra and from 2150 to 2300 nm, for urine spectra, too. In the region from 2040 to 2300 nm the dominated absorption of the N–H and C–H bands connected with proteins. The combination of O–H deformation/C–O stretching vibration around 2100 nm and of O–H stretching/C–O stretching vibration around 2278 nm were associated with carbohydrate absorption. Mastitis decreases lactose content and alters the types of protein in milk. Casein content decreases while whey protein increases. Alteration of the blood–milk barrier leads to an influx of albumin and other blood serum proteins in milk and lactose and milk proteins in blood, such as $\alpha$-lactoalbumin. Altered types and concentration of proteins in milk and blood, and changes in lactose content of milk, blood and urine,[2] explained the differences in the loading of the first PC factors for spectra of healthy and mastitic cows in this spectral region.

## Conclusion

Cow's mastitis diagnosis, based on SIMCA classification of near-infrared spectra of biological fluids, showed similar performance for milk, blood and urine when somatic cell count (SCC) in milk was used as the disease threshold. The best calibration results were obtained with first derivative spectral transformation. They varied from 97.37% to 98.67% for different fluids.

The success of NIR mastitis diagnostics was defined by the effect on the NIR spectra of biofluid compositional changes caused by mastitis.

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