# Classification of Subclinical Mastitis in raw cow milk using Near Infrared Reflectance Spectroscopy

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## ABSTRACT

Mastitis is an infectious-contagious disease that causes an inflammation of the udder that affects a high proportion of dairy cows throughout the world. The difficulty of its diagnosis (which requires culture media especially for its isolation) and inefficiency of antibiotics in your treatment, what become a fearsome enemy if detected its presence in a dairy farm, since only very rigorous hygiene and disposal measures of the positive cows, are the measures known for controlling it. While it is true that subclinical mastitis does not usually increase in greatly the amount of colony forming unit (CFU)/ml (x1000) of tank milk, can contribute some bacteria potentially harmful to human health, also alters the composition of milk. This research introduces the development of an analytical methodology for on-site monitoring the CFU/ml in raw milk at farm level by using a portable NIR sensor MicroPhazir$^{TM}$ NIR spectrometer, using a total of 1266 liquid milk samples, scanned at room temperature without pre-treatment. Samples were divided into two sub-sets. The training set composed of 1197 samples, and a set of 69 samples to external validation. Classification models were used for the prediction of CFU/ml in milk at legal level: $< 400$ and $\geq 400$ CFU/ml(x1000), achieving less than 3% of penalties.

**Keywords:** Cow milk, Subclinical mastitis, NIR sensor, Semi-quantitative classification models

## 1. INTRODUCTION

Several factors can affect bovine mammary gland health, although bacterial mastitis is the most studied and reported cause. Mastitis is an infectious-contagious disease that causes an inflammation of the udder. Mastitis, in particular the subclinical type, is one of the most persistent and widely spread disease conditions of importance to milk hygiene and quality among dairy cattle worldwide.[1] The difficulty of its diagnosis (which requires culture media especially for its isolation) and inefficiency of antibiotics in the treatment, what become a fearsome enemy if detected its presence in a dairy farm. In cows, the somatic cell count (SCC) is a useful predictor of mastitis, the most widely accepted criteria for measuring udder health and milk quality in all major milk-producing countries throughout the world[2] and therefore, it is an important component of milk in terms of hygiene, and mastitis control,[3] because it is a useful indicator for concentration of leukocytes in milk. Elevated milk SCC is associated with altered protein quality, change in fatty acid composition, lactose, ion and mineral concentration, increased enzymatic activity, and a higher pH of raw milk.[1] 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.[4] The total economic losses of mastitis (subclinical and clinical) per cow present in a default situation varied between 65€ and 182€/cow per year depending on the bulk tank somatic cell count and due to its cost,[5] this disease has been the first focus of sensor developments in the dairy sector. The performance of the sensors currently used in practice (mostly based on electrical conductivity of milk), should be improved considerably. Especially the large number of false positive alerts is a concern for many dairy farmers.[4] In this sense, Near

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infrared reflectance (NIR) spectroscopy is a technology capable of covering current analytical applications due to its speed, being nonpolluting, non-destructive and easy to use, and diverse authors have shown the application of NIR technology to milk analysis.[6] The advances in NIRS instrumentation in recent years, the availability of portable NIR instruments, allowed in previous researchers developed a methodology based on the use of hand-held portable NIRS for the analysis of major components (fat, protein and solids-non-fat) in raw milk.[7] Besides, Internet possibilities and remote NIR spectral management joining the development of more powerful and versatile treatment software provided by handheld NIR sensors, and machine learning algorithms, were possible to enable the real-time management and optimisation of the chemical quality control of milk production for each cow individually, through development of a mobile application useful as prediction tool that make it possible to get an in-situ real-time estimation of chemical quality control parameters of individual cow milk at farm level.[8] The objective of this study was to develop an analytical methodology for on-site monitoring SCC as CFU/mL (colony-forming unit/mL) in raw milk at farm level by using a portable NIR sensor to control of mastitis of individual cow milk through the integration of NIR spectral data, provided by a handheld NIR sensor, and machine learning algorithms.

## 2. METHODOLOGY

### 2.1 Milk samples

A total of 1266 liquid milk samples, were collected from individual Holstein-Friesian dairy cows and bulk tank of the experimental farm located in the Regional Institute for Research and Agro-Food Development (SERIDA) under different feeding experiments, and from diverse farms located in the North of Spain (Asturias, Spain) over a longer period from 2015 until 2019, to consider changes in the matrix composition, including temporal changes of the biological material, in order to develop robust models that provide independent results including instrument drifts. The individual milk samples from experimental cows of SERIDA were taken from each cow by using the automatic sampler of automatic milking system (DeLaval, Spain) and in farms by the farmer using different milking systems. From the initial dataset of samples were divided into two sub-sets. The training set used to develop and optimise the model by internal validation was composed of 1197 samples, and a set of 69 samples to external validation.

### 2.2 NIRS and reference analysis

The collection of NIRS spectra data were carry on immediately after milking by using a handheld MEMS digital transform spectrometer (1.8 kg weight) from Polychromix PHAZIR$^{TM}$ (PhIR, Phazir 1624, Polychromix Inc., Wilmington, MA, USA), with a scanning window of 4 mm diameter (sampling area of 0.13 cm2). All diffuse reflectance spectra were computed in a wavelength range between 1600 and 2400 nm, with a non-constant interval of around 8 nm (pixel resolution 8 nm, optical resolution 12 nm) being an hand-held micro-electro-mechanical system (MEMS) digital transform. In this instrument spectra were collected using externally a liquid opaque cuvette (FOSS. Ref US-ISIH-0398), with dimensions of 4.5 cm height, 2.5 cm wide and a 17mm pathlength, with aluminum backside for trans-reflectance measurements (which combines reflectance and transmittance together into a single mode). The samples were homogenized by hand mixing during 20-30 sec and analyzed in triplicate being each spectra the average of 80 scans for collecting one spectra. The final spectrum was the average of all of them. All spectra data were recorded in reflectance mode (log 1/R).

The same portion of the sample used to collect spectra in NIRS instrument was used for reference data analysis of SCC as CFU/mL. Reference analyses were carried out using a Fossomatic instrument (Foss Electric, Hillerod, Denmark) in the Professional Milk and Agro-food Laboratory of Asturias. This laboratory is accredited under UNE-EN ISO/IEC 17025: 2005 (246/LE476). The laboratory error was calculated as repeatability according to ISO 5725[9,10] definitions: (i) repeatability, indicates the variability observed within a laboratory, over a short time, using a single operator, item of equipment etc., employing six bulk tank samples analyzed five times each and calculates according to Eq. 1:

$$r = S_r \cdot 2\sqrt{2} \tag{1}$$

where; r= repeatability; $S_r$= standard deviation of repeatability.

### 2.3 Prediction model

The collected spectral data were converted into a data matrix suitable to be used as the input to a model generator. The X and Y variables were defined as: $X = \lambda$ and $Y = \log \frac{1}{R}$. At first, non-linear models (Neural Networks) and linear ones based on partial least squares (PLS) but they obtained results were far from being successful. They were obtained by applying different pre-treatments, such as the standard normal variate (SNV), multiplicative scatter correction (MSC) and first and second Savitzky and Golay (SG) as derivative mathematical treatments, to the spectral data to quantify SCC using the Unscrambler v. 9.8 application.[11]The final choice was the SNV + first derivative (SG) treatment.

As it is more relevant to determine if the levels are above or below a given legal threshold, a classification approach was followed. In order to generate a dataset suitable to obtain a binary classifier, the Y column was replaced by 0s (CFU/ml $<$ *threshold*) and 1s (CFU/ml $\geq$ *threshold*), where *threshold* was set as 400 CFU/ml which is the legal level in Spain.

To train and validate the classifier, the TensorFlow[12] framework from Google was used. Using this tool a Neural Network-based classifier was defined by using a very simple and well-known topology: a multilayer perceptron with the following structure:

1. Input layer with a size equal to the number of input variables (wavelengths).
2. One hidden layer with a number of neurons half the size of the number of inputs.
3. Output layer with just a single neuron as we are building a binary classifier.

To train the model the binary cross-entropy function was used (Eq. 2), in combination with the Adam algorithm as optimizer.[13]

$$H_p(q) = -\frac{1}{N}\sum_{i=1}^{N} y_i \cdot \log\left(p(y_i)\right) + (1 - y_i) \cdot \log\left(1 - p(y_i)\right) \tag{2}$$

Table 1. Statistic descriptive values for milk samples in calibration and external validation sets.

| | Calibration set (N=1197) | | | Validation set (N=69) | | | Repeatibility of reference method | | |
|---|---|---|---|---|---|---|---|---|---|
| CFU*/ml(x1000) | Range | Mean | SD† | Range | Mean | SD | Range | Mean | SD |
| | 8-2327 | 197 | 381 | 4-2467 | 212 | 441 | 13-49 | 30 | 17.6 |

## 3. RESULTS AND DISCUSSION

In Fig. 1 is shown spectra collected with NIRS handheld instrument after combining scatter correction and mathematical derivation (SNV and 1st derivative). As can be seen in Fig. 1, the strong NIR absorption bands attributed to water due to the hydrogen bonds have led a high value for log (1/R) around 1940 nm (water band), representing the O-H second overtone bending.

In order to test the performance of the classifier obtained in 2.3, a calibration and a validation set were used, as shown in Table 1. This table show values for number of samples, range, mean and standard deviation for the parameters analysed for the calibration and validation sets, as well as for the repeatibility of reference method. Two different tests were carried out: one training the classifier with the calibration set and testing it with the external validation set, and another one using the whole calibration set for cross validation. Table 2 shows the misclassified samples in the first experiment. The overall success rate was 83% and the 95% correspond to

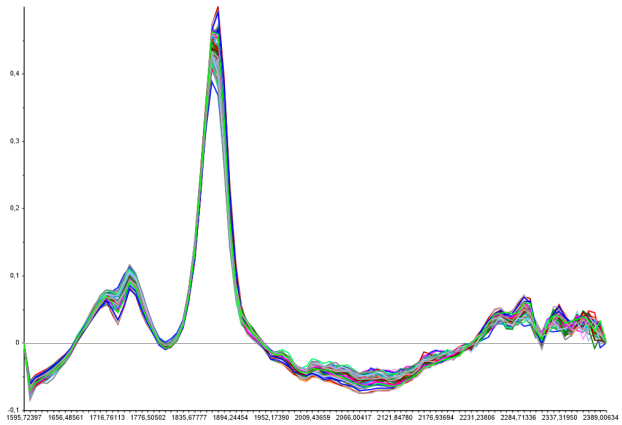
*colony-forming unit/mL

†Standard Deviation

Table 2. Misclassified samples in the first experiment (accuracy=83%).

| Real value | Predicted | Cell count |
|---|---|---|
| 0 | 0.99 | 206.80 |
| 1 | 0 | 1347.00 |
| 1 | 0 | 699.00 |
| 1 | 0 | 1055.00 |
| 0 | 0.99 | 62.00 |
| 1 | 0 | 2467.00 |
| 1 | 0 | 1480.00 |
| 1 | 0.044 | 446.00 |
| 1 | 0 | 654.00 |
| 0 | 0.98 | 193.60 |
| 1 | 0 | 632.00 |
| 1 | 0 | 1725.00 |

Figure 1. Average spectra for milk samples in external validation set measured using a portable NIR sensor MicroPhazir$^{TM}$. Spectra pre-processed by SNV and 1st derivative (SG)



samples from class healthy correct classified. The NIRS estimation achieved a low penalties where threshold was set as 400 CFU/ml.

As the number of samples in the validation set is not very high compared to the size of the calibration set, it was opted to perform a cross validation on that set in order to properly validate the performance of the obtained classifier. In this case the achieved success rate was 100%.

Different research works employing near-infrared (NIR) spectra have established the usefulness of NIRS technology for mastitis diagnosis based on SCC level in milk,[14] developing a "leave-one-cow-out" cross-validation PLS regression model for quantification of somatic cells in milk in the raw milk obtained a moderate correlation (R2=0.76). In this sense[15] employing spectra of quarter for milk of cows and soft independent modelling of class analogy (SIMCA) got 72.15% of specificity and 69.44% of sensitivity using the single threshold method which yielded its best result at 200,000 cells ml-1 SCC level and improved the accuracy with double threshold which had 80.56% of specificity and 77.78% of sensitivity when the healthy and mastitis levels were set at 20,000 cells ml-1 and 200,000 cells ml-1, respectively. However, none of them used a portable instrument as in this work.

Related to the results obtained with the portable analyzer used for healthy quality analysis of raw milk during milking in this work, the classification model shows more optimistic values for detection the presence of subclinical mastitis. In large cow herds, 80% of uninfected animals will have a CFU of less than 200,000 cells/mL

and 50% of less than 100,000 cells/mL. All of them less than 400,000 cells/mL as threshold settled down.

## 4. CONCLUSIONS

The obtained results show the opportunity to use classifiers for the identification of raw milk samples infected with bacteria, ultimate cause for the mastitis in cows in a dairy farm. Near infrared spectroscopy in combination with neural networks setup up for classification, offers an alternative approach to traditional methods for an in-situ and reliable application in bio-diagnostics and food control.

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## REFERENCES

[1] Coulona, J.-B., Gasquib, P., Barnouin, J., Ollier, A., and Pradel, P., "Effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows," *Animal Research - ANIM RES* **51**, 383–393 (09 2002).

[2] Rysanek, D., Babak, V., and Zouharová, M., "Bulk tank milk somatic cell count and sources of raw milk contamination with mastitis pathogens," *Veterinari Medicina* **52** (11 2006).

[3] Harmon, R., "Physiology of mastitis and factors affecting somatic cell counts," *Journal of Dairy Science* **77**(7), 2103 – 2112 (1994).

[4] Hogeveen, H., Kamphuis, C., Wilma, S., and Mollenhorst, H., "Sensors and clinical mastitis—the quest for the perfect alert," *Sensors* **10** (09 2010).

[5] Huijps, K., Lam, T., and Hogeveen, H., "Costs of mastitis: Facts and perception," *Journal of Dairy Science 75 (2008)* **75** (03 2008).

[6] Holroyd, S., "Review: The use of near infrared spectroscopy on milk and milk products," *Journal of Near Infrared Spectroscopy* **21**, 3111 (10 2013).

[7] de la Roza-Delgado, B., Garrido-Varo, A., Soldado, A., González Arrojo, A., Valdés, M., Maroto-Molina, F., and Perez-Marin, D., "Matching portable nirs instruments for in situ monitoring indicators of milk composition," *Food Control* **76** (01 2017).

[8] Muñiz, R., Cuevas-Valdés, M., and [de la Roza-Delgado], B., "Milk quality control requirement evaluation using a handheld near infrared reflectance spectrophotometer and a bespoke mobile application," *Journal of Food Composition and Analysis* **86**, 103388 (2020).

[9] "Accuracy (trueness and precision) of measurement methods and results. part 1: General principles and definitions," standard, International Organization for Standardization (ISO), Geneva, CH (1994).

[10] "Accuracy (trueness and precision) of measurement methods and results. part 2: General principles and definitions," standard, International Organization for Standardization (ISO), Geneva, CH (1994).

[11] Camo Analytics, *Unscrambler v. 9.8*. Camo Analytics, Norway (2008).

[12] Abadi, M., Agarwal, A., Barham, P., Brevdo, E., Chen, Z., Citro, C., Corrado, G. S., Davis, A., Dean, J., Devin, M., Ghemawat, S., Goodfellow, I., Harp, A., Irving, G., Isard, M., Jia, Y., Jozefowicz, R., Kaiser, L., Kudlur, M., Levenberg, J., Mané, D., Monga, R., Moore, S., Murray, D., Olah, C., Schuster, M., Shlens, J., Steiner, B., Sutskever, I., Talwar, K., Tucker, P., Vanhoucke, V., Vasudevan, V., Viégas, F., Vinyals, O., Warden, P., Wattenberg, M., Wicke, M., Yu, Y., and Zheng, X., "TensorFlow: Large-scale machine learning on heterogeneous systems," (2015). Software available from tensorflow.org.

[13] Kingma, D. P. and Ba, J., "Adam: A method for stochastic optimization," (2014).

[14] Tsenkova, R., Meilina, H., Kuroki, S., and Burns, D., "Near infrared spectroscopy using short wavelengths and leave-one- cow-out cross-validation for quantification of somatic cells in milk," *Journal of Near Infrared Spectroscopy* **17**, 345–351 (2009).

[15] Meilina, H., Kuroki, S., Jinendra, B., Ikuta, K., and Tsenkova, R., "Double threshold method for mastitis diagnosis based on nir spectra of raw milk and chemometrics," *Biosystems Engineering* **104**(2), 243 – 249 (2009).