# Comparison of on-line SCC analysers and herd testing for estimating mastitis detection rates

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## Summary

On-line somatic cell count (SCC) analysers automatically test for SCC during milking for individual cows, providing farmers with more measurements than conventional herd testing. The purpose of this study was to determine whether more frequent milk sampling increases detection of elevated SCC due to mastitis. We compared the rate of detection of mastitis events using on-line SCC analysers with that using conventional herd testing with a simulated dataset of 100 random herds of 1,000 cows. Five herd test scenarios (two, four, six, eight, or ten herd-test dates per lactation) were compared to 20 on-line SCC analyser scenarios (varying based on whether cows were milked once or twice daily and the proportion of on-line SCC analysers installed on bails, ranging from 2% (i.e. one in 50 bails) up to 100%). Random mastitis events for each cow were modelled based on composite cow SCC and individual quarter bacteriological data derived from a dataset of 2,345 cow-lactations. We then calculated the average probability of on-line SCC analysers and of herd testing detecting these mastitis events, assuming 280 days of lactation. Herd testing four or ten times per lactation was found to detect 47% or 75% of mastitis cases, respectively. On-line SCC analysers installed on 10% of bails with once-a-day milking detected 84% of cases. We observed that on-line SCC analysers, even on a small proportion of bails, were more likely to detect mastitis events than standard herd testing, in a simulated dataset.

*Keywords: mastitis, on-line SCC analysers, herd testing, automated milk sensors, simulation, somatic cell count, bivariate distribution, copula.*

## Introduction

Analysis of milk from individual cows has traditionally been conducted in off-farm laboratories, using milk samples collected during herd testing. In New Zealand this is typically carried out four times per lactation, or every two months; in Australia and other countries, it may occur eight times per lactation, or monthly. In the last few decades, there has been noteworthy development of small automated devices, such as LIC Automation's CellSense®, which allow direct on-line analysis of milk on farm. These devices enable real-time reporting and detection of trends in somatic cell count (SCC) over the entire lactation as well as more collections and analyses of milk samples (Bewley, 2016).

Mastitis is a costly production disease, causing significant economic loss to dairy industries (Halasa *et al.*, 2007) due to decreased milk production, increased costs of veterinary services and treatment, extra labour, and replacement (Seegers *et al.*,

2003). Clinical mastitis is indicated by signs of disease in the animals or visibly abnormal changes in the milk, but subclinical mastitis is only recognisable through additional testing.

SCC is the standard proxy for subclinical infection. High values indicate mastitis (Moyes *et al.*, 2009). Bulk tank values greater than 400,000 cells/mL are often penalised by dairy companies (Valeeva *et al.*, 2007), while an individual cow threshold of 200,000 cells/mL is commonly used to indicate mastitis (Eberhart *et al.*, 1979; Harmon, 1994). Farmers use cow SCC data to monitor levels of infection in the herd, which informs treatment or culling decisions. Since herd testing in New Zealand typically occurs once every two months, mastitis might not be detected until weeks after the onset of infection. On-line SCC analysers take spot samples during milking, producing an SCC estimate for each cow at the time of milking and providing a more detailed picture of a cow's infection history.

In New Zealand, clinical mastitis is typically caused by *Streptococcus uberis* (SU) or *Staphylococcus aureus* (SA), with other species less commonly isolated (McDougall *et al.*, 2009; Bryan *et al.*, 2011). There are differences in duration, recovery time, and cell count response for each type of infection. *Streptococcus uberis* is found in faecal material, and so is associated more with the environment; intramammary infections are more prevalent during wetter periods, typically near the start and end of lactation. *Staphylococcus aureus* is a contagious pathogen characterised by infections of long duration with a high prevalence observed in late lactation (De Haas *et al.*, 2004).

This is the first study to combine real-world fortnightly and weekly herd test data with periodic bacteriology data from a New Zealand herd to evaluate typical mastitis start times and duration for different pathogen types. An empirical dataset, containing bacteriology data gathered over seven seasons from this well-recorded herd, was used to generate a simulation. Simulations compared the proportion of mastitis events detected using an on-line SCC analyser and using conventional herd testing. The proportions were calculated for all pathogen groups combined as well as separately for the SU and SA groups in order to determine whether pathogen type affected detection rates.

## Materials and methods

### *Empirical data*

The empirical dataset was obtained from bacteriology and SCC records, generated for 2,345 unique cows milked during the 2004, 2005, 2006, 2007, 2010, 2011, and 2013 seasons at DairyNZ Lye Farm (Waikato, New Zealand). Data from 2008, 2009, and 2012 were not included as large numbers of cows were enrolled into mastitis infection challenge studies and the subsequent infections were not representative of natural infections. Any one quarter of a cow's udder can be infected without spreading it to the rest; therefore, milk samples were collected from each quarter. Bacteriology data were determined using standard microbiology methods (Hogan *et al.*, 1999) on quarter foremilk samples. Milk samples were collected aseptically at four times during lactation: at the first milking after calving, at peak-mid lactation, at mid-end lactation and in the last seven days before dry off. Additional samples were collected from affected quarters if clinical mastitis was detected. Cow-lactations were retained in the dataset if sampled all four times, resulting in a dataset of 1,435 cow-lactations and 26,407 quarter samples over all seven seasons. SCC was measured by the routine herd testing, conducted weekly or fortnightly. A total of 30,442 herd test records were available, averaging 21 herd tests per cow-lactation.

Across all seven seasons, 65.99% of the 1,435 cow-lactations were bacteriologically-positive (testing positive at some point for a recognised mastitis pathogen). Retaining only these bacteriologically-positive cows generated a dataset

containing 947 cow-lactations, with 14.32% of quarters being bacteriologically positive at any point in time. The proportion of quarters infected with SU, SA, coagulase-negative *staphylococci* (CNS), and other types of pathogens (other) were 16.41%, 4.55%, 22.9%, and 56.1%, respectively, with the most frequent other pathogen isolated being *Corynebactetrium bovis* (38.31%). This study focused specifically on SU and SA as these are the most common pathogens associated with clinical mastitis in New Zealand and therefore more likely to cause a mastitis event associated with an elevated SCC or clinical episode (McDougall *et al.*, 2007).

Start dates and duration of mastitis events were based on weekly and fortnightly cell count data from herd tests and corresponding bacteriology data for each animal. An inflammatory event was defined as a period when the SCC was above 200,000 cells/mL, starting from the date on which the SCC first exceeded this threshold and ending when SCC dropped below it. A particular inflammatory event was defined as a bacteriologically-positive mastitis event (from hereon referred to as a mastitis event) if a positive bacteriology test occurred within 21 days either side of it. Mastitis events that were $\leq$ 14 days apart were merged into one event, and those at the end of lactation with a duration of < 14 days were arbitrarily assigned a duration of 14 days. When more than one bacterial species was associated with a single mastitis event, the event was assigned a bacterial species in the following order of dominance: SU, SA, CNS, and any other type of pathogen. A total of 790 subclinical and clinical mastitis events were detected over seven seasons: 228 events attributed to SU, 46 to SA, 171 to CNS, and 345 to other pathogens. Three subsets of the empirical data were used to build three separate simulations: i) All bacteria, ii) SU only, and iii) SA only.

## Simulation

All calculations and statistical analyses were conducted using R software version 3.4.0 (R Core Team, 2017). To estimate the detection rate of mastitis by herd testing and by on-line SCC analysers, we simulated 100 herds of 1,000 cows assuming a total of 280 days in milk (DIM) for each cow. Three simulations were conducted, for i) All bacteria, ii) SU only, and iii) SA only. Individual mastitis events from the empirical data were taken as independent data points when fitting the marginal distributions. The distribution of DIM when mastitis was first detected was approximated by a distribution obtained from the sum of a gamma distribution (for the first half of the data) and a normal distribution (for the second half). A gamma distribution was also used to approximate the distribution of the duration of mastitis (except when modelling SA, for which the sum of a gamma distribution and a uniform distribution was used). Parameters for the marginal distributions were estimated based on the data using the fitdistr function in the MASS R package (Venebles and Ripley, 2013).

Plotting the data indicated non-independence of the duration of mastitis and the DIM when mastitis was first detected. Therefore, it was deemed more appropriate to sample from a bivariate joint distribution model than from the two marginal distributions independently. Copulas were used to characterise the dependence structure between the two marginal distributions. The BiCopSelect function from the VineCopula R package (Schepsmeier *et al.*, 2012) was used to determine the best-fitted copulas: Student t for SA and Frank for SU and all bacteria. We sampled from the resulting bivariate joint distributions to produce one single mastitis event during each cow's lactation (restricted to 280 days) in the 100 simulated herds.

Herd test dates for the 2015 season (7,267 farms) were extracted from the LIC herd recording database to simulate four herd test dates for each of the 100 simulated herds. The dataset was restricted to farms with four test dates and a first calving date between 2015-07-01 and 2015-08-31, reducing the number of farms in the dataset

to 2,895. To simulate herd test dates, we sampled from four uniform distributions centred around the four mean dates for each of the first to fourth herd tests. To simulate routines with more herd tests, uniform distributions were built around equally-spaced centres. The length of the range of each uniform distribution was 20% of the interval between adjacent centres.

Farmers may choose to install on-line SCC analysers in only a subset of bails. Therefore, we modelled on-line SCC analyser events for a range of proportions of bails with analysers (p). We assumed that each cow was equally likely to be milked at any bail on any given day. The probability of any given cow being milked at a bail with on-line SCC analysers on a particular day (P) was calculated as $P = p$ for cows milked once a day, or $P = 1 - (1 - p)^2$ for cows milked twice a day. On-line SCC analyser events (the analyser taking a sample at milking) were generated for each cow and each day when $U < P$, where U was independently sampled for each event from a random uniform distribution (range 0 to 1).

### *Obtaining simulated detection rates*

Each cow in each simulated herd was modelled to have a single mastitis event during its 280 days of lactation. We then simulated two, four, six, eight, and ten herd tests for each herd and on-line SCC analyser events for one or two milkings, where p = 0.02, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.50, 0.75, or 1.00. The average mastitis detection rates for all herds and animals were calculated for all herd test and on-line SCC analyser scenarios. Each of the three simulations used separate distributions of mastitis start dates and duration, based on each of the bacteria classes: i) All bacteria, ii) SU, and iii) SA.

## Results

The duration of mastitis events was found to be dependent on the bacterial species and the DIM when they were first detected (Figure 1). Therefore, three bivariate distributions (duration and DIM) were designed for the three subsets of the empirical data: i) All bacteria, ii) SU only and iii) SA only.

The plots for the all bacteria simulation are shown in Figure 2. The parameters for the marginal distributions and the best-fitting copulas were selected based on maximum likelihood. The distribution of DIM (Figure 2A) was modelled as the sum of a gamma (shape =1.042, rate = 0.022) and a normal distribution (mean = 228.7, sd = 41.95). The distribution of duration (Figure 2B) was modelled as a gamma distribution (shape = 0.85, rate = 0.024). The empirical (Figure 2C) and modelled (Figure 2D) bivariate distributions (using a Frank copula (par = -0.35, tau = -0.04)) show that shorter mastitis events were concentrated at the start and end of lactation. The same process was applied to data based on SU infections only and SA infections only to create bivariate distributions based on a Frank copula (par = 1.64, tau = 0.18) for SU and a Student t copula for SA (par = -0.58, par2 = 3.01, tau = -0.4), respectively.

Within each simulation, the average detection rates were compared between herd testing scenarios and different on-line SCC analyser scenarios (Figure 3). As expected, detection rates improved with increasing frequency of herd tests. Similar detection rates were achieved for all bacteria and SU-only simulations and higher rates for SA-only simulations at all frequencies of herd testing. Mean detection rates for herd testing were 58%, 57%, and 73% for all bacteria, SU-only, and SA-only simulations, respectively.

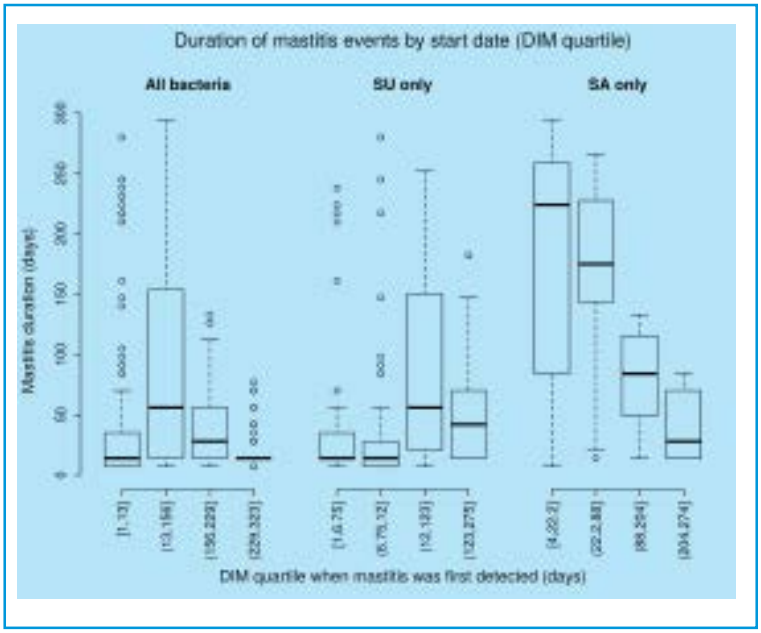


*Figure 1. The relationship between mastitis duration and the DIM quartile when mastitis was first detected.*

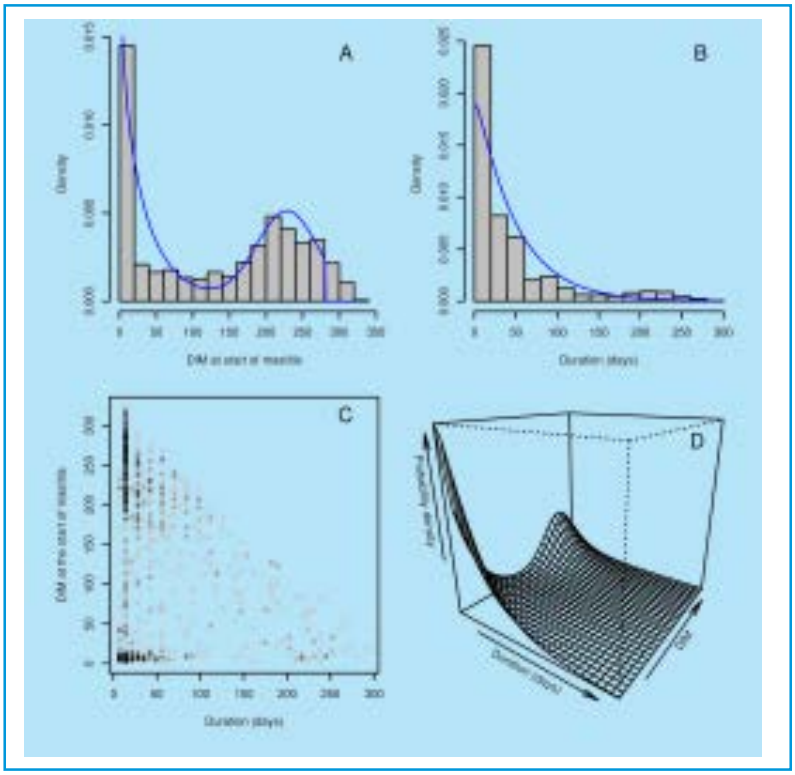


*Figure 2. Plots for 'all bacteria' simulation: A) Marginal distribution of DIM when mastitis was first detected (grey bars) and the fitted distribution (blue line: sum of gamma and normal distribution); B) Marginal distribution of duration of mastitis and the fitted distribution (blue line: gamma distribution); C) Scatterplot of the relationship between DIM at the start of mastitis and duration; D) 3D density plot of the probability density function of the bivariate distribution generated using a Frank copula and the marginal distributions.*

For on-line SCC analysers, higher detection rates were achieved in simulations of a higher proportion of bails installed, and of cows milked twice instead of once a day (Figure 3). As for herd testing, detection rates were similar for all bacteria and SU-only events, and higher rates observed for SA-only, with mean detection rates of 86%, 86%, and 92% respectively observed for the different simulations.

On-line SCC analysers installed on 2% of bails with once-a-day milking produced a similar detection rate to four herd tests for the all bacteria simulation (46.6% and 47%, respectively). With on-line SCC analysers installed on 10% of bails (still operated with once-a-day milking), a detection rate of 84% of all bacteria events was achieved, significantly higher ($P < 0.001$) than the detection rate of 75%, achieved with ten herd tests.

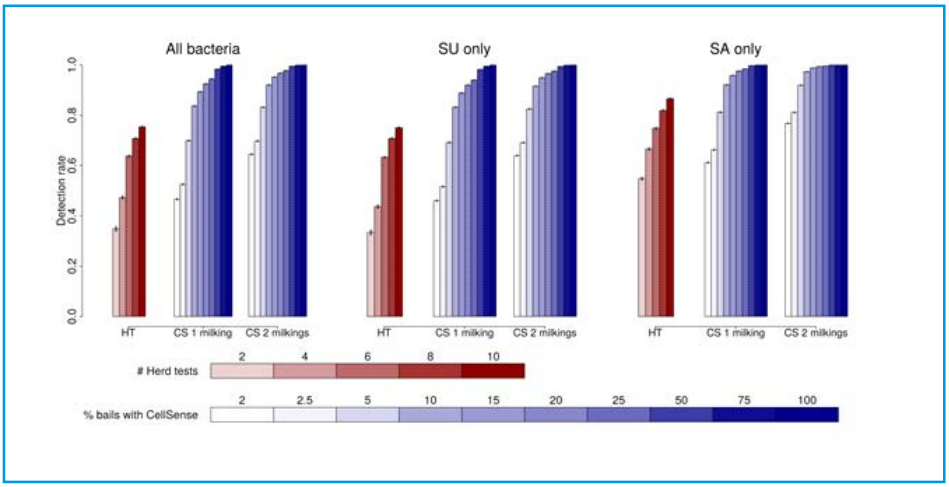


*Figure 3. The average mastitis detection rate for different scenarios of herd testing and on-line SCC analysers calculated from 100 simulated herds of 1000 cows. Error bars represent 95% confidence intervals.*

## Discussion

This is the first New Zealand study to compare on-line SCC analysers and herd testing in detecting SCC events associated with mastitis. Using our modelling approach, we observed that an on-line SCC analyser approach was far more likely than conventional herd testing to detect individual mastitis events, due to the more frequent measurement of SCC. Mean detection rates of 86% and 58% were achieved by on-line SCC analysers and herd testing respectively for all bacteria. On-line SCC analysers installed on at least 10% of bails were more likely to detect mastitis events than any of the simulated scenarios of herd testing. On-line SCC analysers installed on just one out of 50 bails for once-a-day milking had detection rates comparable to four herd tests (both 47%).

This study illustrated a method for simulating mastitis events by using copula functions to link the marginal distributions of DIM when mastitis was first detected and mastitis duration to generate bivariate joint distributions. This bivariate copula model could be extended to become multivariate copulas, incorporating more parameters, which may make simulations more realistic. Copulas are widely used in a variety of disciplines, and their use has developed considerably in recent years (Salvadori and De Michele, 2007; Genest *et al.*, 2009; AghaKouchak *et al.*, 2010). They have been used to model mastitis in dairy cattle; for example, correlated infection times in the four udder quarters of cows have been modelled using four-dimensional copulas (Massonnet *et al.*, 2009;

Prenen *et al.*, 2017). Copulas are suitable for modelling complex biological systems where it is difficult to describe the dependency structure of the joint distribution. Their usefulness in modelling mastitis in dairy cattle should be explored further.

The epidemiology of mastitis varies between pathogen types (Zadoks *et al.*, 2011). It is for this reason that we built separate simulations for three pathogen groups. Indeed, prevalence, timing, and duration of mastitis differed depending on the type of infection. Cows infected with SU were more likely to develop mastitis earlier in lactation for shorter episodes, whereas mastitis in SA cows was more often contracted at the start and persisted to the end of lactation.

This study reported the combined prevalence of subclinical and clinical mastitis. The prevalence of SU in this herd was found to be higher than SA, consistent with previous reports from New Zealand herds (Compton *et al.*, 2007; McDougall *et al.*, 2009; McDougall *et al.*, 2010; Pankey *et al.*, 1996), although exceptions where prevalence of SA exceeds that of SU have also been reported (Bryan *et al.*, 2011). Subclinical and clinical mastitis were not modelled separately in the study due to a lack of available data; however, this would be a potentially interesting approach for future study should such datasets become available.

The prevalence of SA was lower in this herd (4.55% out of bacteriologically-positive quarters) than expected, based on the previously reported prevalence of clinical mastitis during lactation, estimated to be 10% among bacteria-positive glands in one New Zealand study (McDougall *et al.*, 2009). Good management practices on this farm may have resulted in a low prevalence of SA, resulting in a low contagious spread at milking time. The low prevalence of SA led to a scarcity of available data points, resulting in a less precise model for simulation compared to other types of infection.

For simplicity, only one mastitis event was allowed for each simulated cow-lactation; realistically, multiple events can take place, which should be explored in future simulation studies. Additionally, simulations were based on mastitis-positive simulated cows only; future simulations could model herds with both infected and uninfected cows based on the incidence rate in the empirical data. It was also assumed that SCC was elevated every time an animal became infected, which may not be so in all circumstances; for example, cows with weakened immune systems for a period post-calving may not respond with elevated SCC (Zadoks, 2006).

Some mastitis events were not observed for the full event time (right censoring); that is, some animals were lost from the herd, or dried off, or data collection ended before the mastitis event ended naturally. In these cases, total duration for an arbitrary 14 days was assumed. Durations in this study may be underestimated (especially near the end of lactation), and further investigation is required. The term “detection rate” was used to describe the proportion of on-line SCC analyser events or herd test events occurring simultaneously with simulated mastitis events. However, simulation did not take into account accuracy or bias of the two measurement methods; this should be incorporated into future simulations.

Mastitis is a multifactorial disease, and any modelling will inevitably simplify the biological system. In this study, we obtained sampled mastitis events directly from a bivariate distribution approximated using the empirical herd test and bacteriological data from a single farm in New Zealand. This approach captures the complexity of the disease by emulating real-world data. However, the restriction to a single farm as a basis for estimated mastitis parameters and distributions may make the study less applicable to other dairy herds due to variation in mastitis circumstances between farms in terms of climate, herd, and management (Hogeveen *et al.*, 2011). Also, assumptions were made where the literature did not fully describe certain parameters:

the minimum number of days between periods of high SCC for an event to be distinct, the window of time during which bacteriology tests were effective, and the order of dominance of bacterial species.

The time of mastitis detection affects the effectiveness of mastitis control at the herd level, as infections early in lactation can incur higher economic cost than later ones (Rollin *et al.*, 2015). Through increased frequency of testing, on-line SCC analysers are more likely to enable faster and earlier detection of mastitis events and, thus, earlier treatment and recovery or removal from the herd, ultimately reducing financial impact from the disease.

## Conclusion

This is the first study to compare the potential detection rate for mastitis using on-line SCC analysers with herd testing. The results from this study demonstrate that installing on-line SCC analysers on even a small proportion of bails is more likely to allow detection of mastitis events than standard herd testing for all pathogen groups studied. Detection rates for both on-line SCC analysers and herd testing were higher in the SA pathogen group than in the all bacteria and SU pathogen groups, due to the persistence of SA infections. Future work could explore the economic and production benefits of on-line SCC analysers in providing SCC trends and earlier detection of mastitis.

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