Comparison of individual cow SCC estimates using an on-line SCC analyser and conventional herd tests

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Summary

The ICAR Recording Guidelines include provision for on-line milk analysis. However the guidelines are inconsistent with some of the practical realities of on-line milk analysis. Consequently, no milk analyser has been able to achieve ICAR approval to date. In the case of somatic cell count (SCC), the use of percentage error limits at low SCC makes compliance practically unattainable. More generally, the guidelines do not recognise the importance of cow-specific bias (CSB), which should be the main focus of technology evaluation for on-line milk analysers. This study investigated the level of CSB exhibited by a commercially available on-line SCC analyser. CellSense® obtained on-line SCC results at each cow milking. Conventional laboratory SCC results were obtained from herd tests conducted at twenty consecutive milking sessions (i.e. ten days). CellSense and herd test SCC results were compared in two ways: paired single samples; and cow-averaged samples. For context, cow-mean herd test results from one day were compared with cow-mean herd test results from ten days. CellSense provided a better estimate of ten-day cow-mean SCC than a single-day herd test. Yet this technology falls well short of the accuracy limits for SCC analysers specified in the ICAR Guidelines. This is a compelling demonstration that the ICAR accuracy limits for on-line SCC analysers need to be reviewed.

Keywords: on-line milk analysers, CellSense, mastitis, somatic cell count, cow-specific bias.

Introduction

ICAR has recognised the increasing use of on-line milk analysers and their potential to undermine farmer participation in official milk recording programmes. Accordingly, the ICAR Recording Guidelines include provision for on-line milk analysis (ICAR, 2017a and b). To date, no milk analyser has attained ICAR approval. This is partly because the ICAR guidelines for on-line milk analysers are inappropriate, given the practical realities of on-line milk analysis. In the case of SCC specifically, the use of percentage error limits at low SCC makes compliance practically unattainable. A low SCC sample can have a large percentage error at a level that is inconsequential for mastitis management or animal evaluation. Another area of concern is that the guidelines fail to recognise the importance of cow-specific bias (CSB) in technology evaluation. CSB occurs when a measurement is consistently under- or overestimated for a given cow relative to the rest of the herd (Anderson *et al.*, 2016). The random component of measurement error will average out with multiple measurements, whereas the cow-specific component will not. CSB therefore limits the ability to

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accurately rank animals within a herd, which is crucial for animal evaluation. It is this aspect of on-line milk analysers that should be the main focus of technology evaluation, and was the subject of this study.

In order to overcome some of the practical constraints of on-line milk analysis, CellSense does not collect a representative proportional sample. Instead it sucks a 'spot-sample' from the milk tube at a predetermined time during the milking (Whyte *et al.*, 2004). This sampling method is acceptable for detecting and monitoring mastitis, but can be substantially different to the SCC measured from a proportional representative milk sample. If these differences contribute to CSB, they will limit the ability to obtain a good estimate of cow-mean SCC from the analyser over multiple measurements.

The aims of this study were to:

- Test whether the differences introduced by CellSense's sampling method are cow-specific or random.
- Evaluate the ability of CellSense to estimate the short-term cow-mean SCC, and ultimately whether CellSense is suitable for the purpose of animal evaluation.
- Review the suitability of the current ICAR error limits for SCC analysers.

Materials and methods

Data were collected from equipment installed at Livestock Improvement Corporation's Innovation Farm, Rukuhia, New Zealand during the 2014-2015 milking season. The milking herd was 345 spring-calved cows, milked twice per day in a 34-bail rotary milking system. CellSense on-line SCC analysers (LIC Automation, Hamilton, New Zealand) were installed at 17 milking positions (50%), measuring SCC from individual cows at each milking. An earlier version of this technology was reported by Whyte *et al.* (2004). Herd tests were conducted at twenty consecutive milking sessions, spanning a ten-day period from the afternoon of 2 November until the morning of 12 November 2014. Samples were collected using Tru-Test Wide Bore Field Collection mechanical meters (Tru-Test, Auckland, New Zealand). The samples were analysed for SCC using flow cytometry at LIC Sample Processing North (Hamilton, New Zealand).

Cows with greater than four CellSense results in the period, and with two herd test results on 7 November were included in the analysis (259 cows). The distribution of tests per cow is shown in the histograms in Figure 1. All SCC averages described in this paper were calculated using the geometric mean. No attempt was made to adjust for AM-PM differences. CellSense results were compared with herd test SCCs for individual measurements (Single-test Comparison) and after averaging the results for each cow (Aggregated Comparison). For the Aggregated Comparison, all available herd test results in the period were averaged, by cow, to produce aggregated herd test SCC values for each cow. These were the gold standard cow-mean SCC values for the period. CellSense results were processed in the same way to produce aggregated CellSense values for each cow. These were the CellSense estimates of cow-mean SCC for the period. To put these comparisons in context, the 7 November (middle day) cow-mean herd test SCC was compared with the ten-day cow-mean herd test SCC (Herd-test Comparison).

Performance was quantified in three ways. First, the correlation (r) between $\log_2$ SCC estimates was calculated. Second, the animal SD (accuracy) defined in the ICAR Guidelines (2017a and b) was determined by calculating the standard deviation of relative error (SDRE) for cows or samples with herd test SCC values less than or

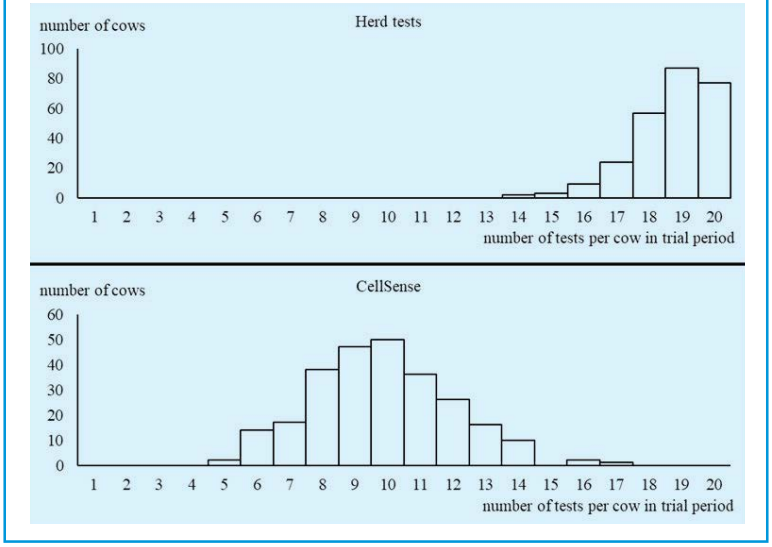

Figure 1. Histograms showing the distribution of tests per cow in the trial period for herd tests (upper) and CellSense (lower).

equal to 2000 kcells/mL (ICAR Method). To calculate the SDRE, the difference between CellSense and herd test values was first calculated. This difference was then divided by the herd test value for that cow or milking to obtain the relative error. The sample standard deviation of relative errors across all cows or milkings was the SDRE. Third, the data were divided into two groups according to herd test SCC. Cows or milkings with SCC less than 200 kcells/mL were in the low SCC group, for which the standard deviation of error (SDE) was calculated. SDE was calculated as the sample standard deviation of the differences between CellSense and herd test SCC values. For the remaining cows or milkings in the high SCC group, the SDRE was calculated (Banded Method).

Comparisons between herd tests and CellSense for SCCs outside the range 100-1500 kcells/mL are problematic for a number of reasons discussed later in this paper. Therefore the analysis was repeated after bounding all individual SCC results within the range 100-1500 kcells/mL. In this case, SCCs lower than 100 kcells/mL were set to 100 kcells/mL, and those greater than 1500 kcells/mL were set to 1500 kcells/mL.

Results

The plots in Figure 2 and statistics in Table 1 illustrate the effect of aggregating SCC data by cow. The correlation of the Single-test Comparison was 0.493, which improved to 0.732 in the Aggregated Comparison and 0.929 with bounding. This correlation was similar to the (unbounded) Herd-test Comparison (0.942). Bounding the data for the Herd-test Comparison did not improve its correlation (0.914). Using the ICAR method, the SDRE values for the Single-test Comparison (548%) and Aggregated Comparison (165%) were very large. The SDRE for the Herd-test Comparison (57%) was also well outside the ICAR limit of 25%. Using the Banded Method, the SDE and SDRE improved from 51.6 kcells/mL and 46.0% in the Single-test Comparison to 33.6

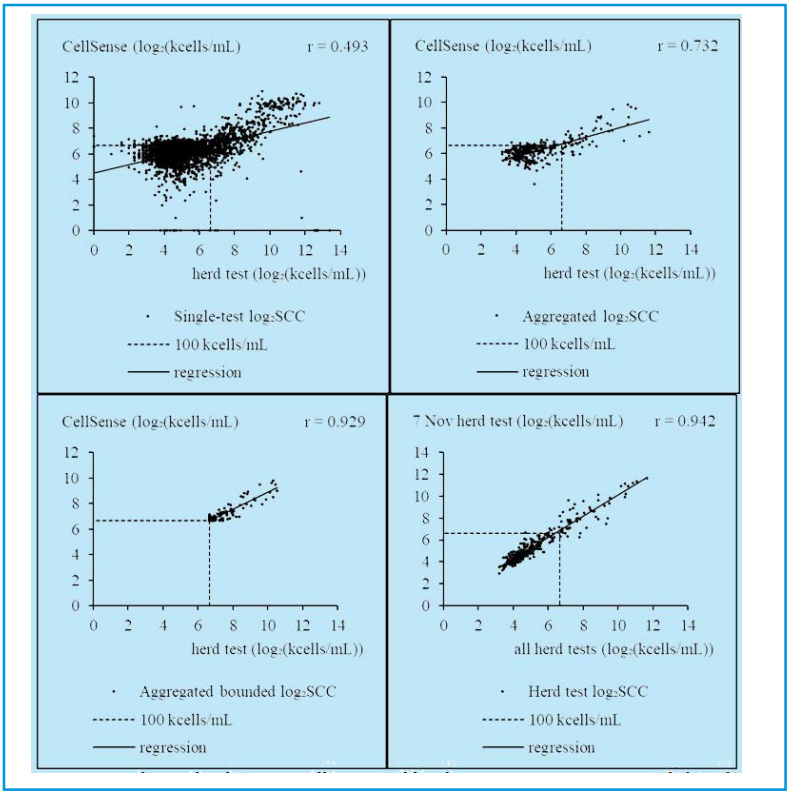

Figure 2: Relationship between CellSense and herd test SCC estimates. Upper left: individual milking results. Upper right: aggregated ten-day mean SCC. Lower left: aggregated ten-day mean SCC after bounding. Lower right: single-day aggregated herd test versus ten-day aggregated herd test.

Table 1. Performance metrics for each comparison method. The number of cows or tests included in the statistic is shown in parentheses.

	Correlation (r)	SDRE	SDE (kcells/mL)	SDRE
SCC range (kcells/mL)	All	0-2000	<200	≥200
Single-test comparison	0.493 (2332)	548% (2293)	51.6 (2042)	46.0% (290)
Aggregated comparison	0.732 (259)	165% (257)	33.6 (227)	29.5% (32)
Bounded aggregated comparison	0.929 (259)	17%[1] (259)	12.0[1] (224)	24.5% (35)
Herd-test comparison	0.942 (259)	57% (257)	64.1 (227)	55.9% (32)
Bounded herd-test comparison	0.914 (259)	34%[1] (259)	50.5[1] (224)	55.4% (35)

[1]These statistics are distorted from bounding low SCC results to 100 kcells/mL.

kcells/mL and 29.5% in the Aggregated Comparison for the low and high SCC groups respectively; and in the Herd-test Comparison, the SDE and SDRE for the low and high SCC groups were 64.1 kcells/mL and 55.9% respectively.

Discussion

There are two primary sources of systematic CellSense error compared with a herd test. First, the on-line analyser uses a different measurement principle to the flow cytometry used by laboratory SCC analysers. Flow cytometry involves staining the DNA of somatic cells and counting the number of stained DNA particles, whereas the detergent used by the on-line analyser lyses somatic cells and interacts with the unwound DNA molecules to increase the viscosity (Whyte *et al.*, 2004). We hypothesise that the differences between the two methods would be clearest at low SCC, where dead epithelial cells constitute the largest proportion of the total SCC. The second primary source of error is the spot-sampling method used by CellSense, because SCC is known to vary markedly within a cow milking (Sarikaya and Bruckmaier, 2006). Error from this source would be greatest at high SCC, where SCC fluctuates the most within a cow-milking. Although both sources of error could produce CSB in theory, the results of the current study show that CSB is relatively small for CellSense as evidenced by the marked improvement in performance metrics after aggregation.

Until now, the primary use of an on-line SCC analyser has been for mastitis management. A useful on-line SCC analyser will perform well in the 100-1500 kcells/mL range. This allows subclinical mastitis to be detected and provides a measure of severity (DairyNZ, 2012). The same rationale can be applied to laboratory SCC analysers. Indeed, laboratory instruments such as the Fossomatic 7 do not claim accuracy outside the range 100-1500 kcells/mL (Foss, 2017). Animal evaluation schemes use a log transformation for all SCC computations (e.g. DairyNZ, 2017a). Using the log scale has advantages, but highly exaggerates errors at low SCC. This is particularly alarming, given that most cows have SCC less than 100 kcells/mL (69% of all NZ cow tests in the 2016-2017 season, from unpublished data). The implication is that the majority of cows are evaluated on the basis of samples with SCC outside the claimed Foss accuracy range, at an SCC level where errors are exaggerated on the $\log_2$ scale. Despite this, continued improvement in SCC attributed to genetic gain has been observed (e.g. DairyNZ, 2017b). This suggests it is the differentiation between animals with SCCs greater than 100 kcells/mL that is driving genetic gain, and that there is little value in accurately ranking cows with SCC less than 100 kcells/mL.

There are also implications for the evaluation of on-line SCC analysers. The animal SD (accuracy) defined in the ICAR Guidelines (2017a&b) surprisingly stipulates the use of SDRE even for samples with 0 cells/mL. At low SCC, the relative error is highly exaggerated, resulting in inflated SDRE values. Most samples fall outside the claimed accuracy range of the reference method (79% of milkings in the current study), at an SCC level where relative errors are exaggerated. There is therefore a strong risk of new SCC technology being unfairly disadvantaged. Rather than use SDRE as the performance metric across the whole SCC range, it would be fairer to use the Banded Method, with SDE at low SCC and SDRE at higher SCC, similar to the ICAR error limits for milk yield (ICAR, 2017a).

The evidence from the present study exemplifies this. When evaluated using the correlation on the log2 scale and eliminating the influence of errors at low SCC by bounding the data, CellSense provided an estimate of the cow-mean SCC in the ten-day period with similar accuracy to a single-day herd test. When evaluated using

the Banded Method, CellSense provided a substantially better estimate of the cow-mean SCC in the period than a single-day herd-test. From these analyses, we conclude that CellSense is suitable for animal evaluation purposes, exhibiting minimal CSB.

The advantage of CellSense is even greater given its ability to monitor cows across the entire lactation. A mastitis event is far more likely to coincide with a CellSense test than an occasional herd test (Zhang *et al.*, 2018). Figure 3 shows one of the more extreme cows in the current trial. This example shows the 7-November herd test coinciding with a brief mastitis event, and causing a very large error in the single-day herd test estimate of cow-mean SCC. It is easy to envisage the opposite occurring, with the herd test missing the mastitis event altogether. The high day-to-day variation typically exhibited by SCC, limits the ability of a single-day herd test to estimate the true cow-mean SCC, while the frequent measurements from analysers like CellSense do account for day-to-day variation. This is one reason why CellSense can provide a better estimate of the ten-day cow-mean SCC than a single-day herd test.

Despite the compelling evidence in favour of CellSense, when evaluated according to the current ICAR Guidelines, CellSense was 22 times worse than the ICAR error limit. The poor performance when measured in this way was mainly due to the exaggeration of small errors at low SCC (the majority of milkings), and does not reflect the ability of CellSense to detect the presence and severity of mastitis.

Conclusions

CellSense provided a better estimate of ten-day cow-mean SCC than a single-day herd test. This implies that the errors due to CellSense's sampling method are sufficiently random and CSB sufficiently small, that CellSense is suitable for animal evaluation purposes. Yet this technology falls well short of the accuracy limits for SCC analysers specified in the ICAR Guidelines. The findings from this trial demonstrate that the ICAR accuracy limits for SCC need to be reviewed. The performance metric for SCC error limits should not exaggerate errors at low SCC, as the current metric does. One option could be to use SDE at low SCC and SDRE at higher SCC, as demonstrated in this trial. Results should also be averaged by cow as part of the evaluation to provide an estimate of CSB.

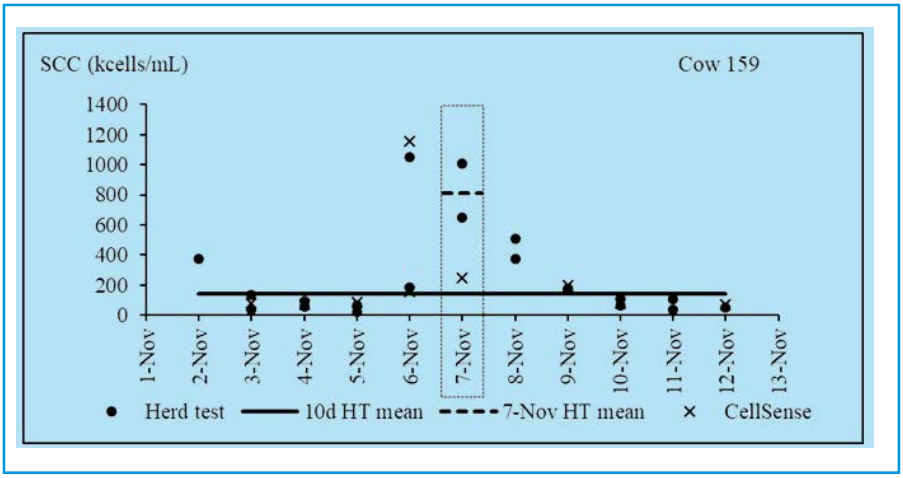

Figure 3. Example SCC results for an individual cow with a large difference between single-day and ten-day herd test values. The dashed box highlights 7 November, which was the single day chosen for the Herd-test Comparison.

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