



Estimation of individual somatic cell counts using single nucleotide polymorphisms from bulk milk samples

A. Pichlmeier,^{1*} L. Ziegler,² Y. Zablotzki,¹ F. Onken,³ M. Iwersen,¹ H. Zerbe,¹ and R. Martin¹

¹Clinic for Ruminants with Ambulatory Services and Herd Health Management, Faculty of Veterinary Science, Ludwig Maximilian University of Munich, 85764 Oberschleissheim, Germany

²LKV Genocell GmbH, 73230 Kirchheim unter Teck, Germany

³German Association for Performance and Quality Testing, 49324 Melle, Germany

ABSTRACT

The SCC of individual cows plays an important role in the assessment of udder health. Until now, individual milk samples have been required to determine the SCC of each cow. This study introduces a novel measurement method that uses the genetic information of individual cows to identify them within a shared bulk milk sample. The Genocells method applies DNA chip technology and genotyping data originally generated for breeding purposes. Using this approach, individual SCC values for all cows in a herd can be estimated from a single bulk milk sample. The first part of the study investigated how many bulk milk samples are required to obtain reliable individual SCC estimates. In the second part, results obtained with the Genocells method were compared with SCC values from individual milk samples collected during routine monthly milk recordings. The Genocells method showed no significant differences in an intra-assay comparison, in which 10 samples from the same bulk milk were analyzed. When compared with SCC results from individual milk samples, significant differences were observed only when SCC values were below 100,000 cells/mL. Overall, Genocells results showed a strong correlation with individual sample results, with 81.5% of values assigned to the same SCC class as those obtained from individual milk samples collected on the same day. Because of the substantially reduced sampling effort, the Genocells method represents a promising approach for monitoring individual udder health in dairy herds. The ability to derive individual SCC values for all cows from a single bulk milk sample constitutes a clear advantage over conventional methods that rely on individual sampling.

Key words: individual somatic cell count, genetic analysis, bulk milk sample

INTRODUCTION

Good udder health monitoring is considered a key factor for successful dairy production. In addition to determining the incidence of clinical mastitis and detecting specific microbiological pathogens causing mastitis, SCC analysis is an important parameter for monitoring udder health in dairy herds. The SCC of individual cows is used as an indicator of the level of inflammation present, whereas the SCC of a dairy herd serves as a parameter reflecting general udder health within the herd. Elevated SCC levels represent unused performance potential, as cows with higher cell counts typically have lower milk yields (Hand et al., 2012; Cinar et al., 2015; Chen et al., 2021) and poorer reproductive performance, including lower conception rates and an increased risk of abortion (Pinedo et al., 2009; Löf et al., 2014; Barbosa et al., 2018). It is therefore important to monitor SCC levels of individual cows frequently to recognize rising SCC levels as early as possible, because early treatment has a higher likelihood of success (Kortstegge et al., 2024).

The first tool for SCC measurement was manual cytology, which has high accuracy but is also time-consuming and expensive (Sun et al., 2023). In Germany, most dairy cows are monitored through monthly milk recordings (MR), using an individual milk sample from each cow to determine milk components such as fat, protein, urea, and SCC by flow cytometry (FC). This method is based on the characteristic light scattering of fluorescent-labeled somatic cell populations (Redelman et al., 1988; Rivas et al., 2001; Feng and Zheng, 2005). Reported correlations between FC and microscopic methods range from $r = 0.90$ to 0.91 (Feng and Zheng, 2005) to $r = 0.97$ to 0.99 for preserved and refrigerated milk (Gonzalo et al., 2003). However, if milk is not preserved and stored at room temperature, correlations are significantly lower ($r = 0.71$ – 0.92 ; Gonzalo et al., 2003). Although both methods are therefore comparable (Feng and Zheng, 2005), they share disadvantages, including high costs per sample because of the need for individual samples and the risk

Received January 3, 2026.

Accepted April 13, 2026.

*Corresponding author: a.pichlmeier@med.vetmed.uni-muenchen.de

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

of confounding intact cells with cytoplasmic granules or dead cells (Sun et al., 2023). Another well-established method is the California Mastitis Test, which is more practicable in a farmer's daily routine; however, it only indicates tendencies and does not provide accurate SCC values (Huang and Kusaba, 2022; Sun et al., 2023).

To reduce time demands and administrative effort for farmers, a new method has been developed that allows determination of SCC for individual cows by genetic analysis of a single bulk milk sample. Provided that all cows in the herd are genotyped, somatic cells within the bulk milk can be differentiated using a DNA microarray technique. For individual differentiation, it is not necessary to know the entire genomic sequence of each cow, but only several thousand SNPs, representing substitutions of a single nucleotide at specific genomic positions. As cows, similar to humans, have a diploid genome, 3 possible combinations exist for each SNP. These can be visualized using fluorescent-labeled target sequences and converted into numerical values. Previous studies have shown that a DNA chip containing 50,000 SNPs is sufficient to distinguish between up to 100 cows (Blard et al., 2012). This approach results in a list indicating the percentage of DNA each cow contributes to the bulk milk sample. When this percentage is related to the individual cow milk yield within the bulk milk, the SCC of each cow can be calculated from the bulk milk sample. This method is commercially known as Genocells. The objective of the present study is to compare the Genocells method with the standard FC method based on individual milk samples under practical conditions.

MATERIALS AND METHODS

To verify the reproducibility of the Genocells method, an intra-assay trial with repeated measurements was conducted. On a farm with 60 cows, 10 bulk milk samples were taken within 5 min from the same bulk tank. In a second step, Genocells results were compared with FC results from monthly MR data obtained from 4 separate dairy farms in southern Germany. The farms had herd sizes ranging from 62 to 85 cows and used freestall housing systems. The predominant breeds were Simmental and Brown Swiss. Cows were milked twice daily in milking parlors equipped with automatic milk yield measurement. Farmers were instructed to collect one bulk milk sample per month over a 1-yr period on the same day that MR sampling took place. Because of operational issues, such as time pressure during harvest season or prolonged transport times, only 36 bulk milk samples could be analyzed using the new genotyping method. These results were compared with data from 1,636 individual milk samples collected from 354 cows.

Bulk milk samples were collected after all cows in the herd had been milked at least once on the respective day. Before milk collection, the storage tank was cleaned to minimize the risk of cross-contamination with previously collected milk. After completion of milking, the bulk milk was stirred for 3 min to ensure homogeneity. The first 2 to 3 L of milk were discarded to remove any potentially unmixed milk. Subsequently, ~20 mL of bulk milk was collected and stabilized by mixing with 100 µL of dried Bronysolv in milk sample containers (Alfred Schiechel ANA.LI.TIK. e.U., Austria; 90.062.005). Samples were stored in a cool, dry place until collection by postal service, with shipping times ranging from 2 to 6 d. To ensure adequate sample quality after prolonged transport, the pH value was measured upon arrival at the laboratory, and samples with a pH below 6.40 were excluded from further analysis. Upon arrival at the laboratory of Milchprüfing Baden-Württemberg e.V., samples were heated to 40°C. One milliliter of milk was transferred to a centrifuge tube and centrifuged at room temperature for 2 min at $500 \times g$ using a ThermoFisher Scientific MicroCL 21 centrifuge. From the resulting sample, 200 µL were collected from the bottom layer and used for genome extraction. The remaining milk was used to determine the SCC of the bulk milk sample using an FTS/FCM 500 combi analyzer (Bentley Instruments, MN). The DNA extraction was performed using the NucleoMAG Tissue Kit (Macherey-Nagel), following the manufacturer's standard protocol. Briefly, 200 µL of milk was mixed with 25 µL SDS/proteinase K solution (buffer T1) and 200 µL lysis buffer (T1) and centrifuged at room temperature for 15 s at $1,500 \times g$. Samples were incubated for 1 to 3 h at 56°C to achieve complete lysis. After centrifugation at room temperature for 5 min at $5,600 \times g$, 225 µL of the lysate was transferred to a square-well block. Subsequently, 24 µL NucleoMag B-Beads and 360 µL binding buffer (MB2) were added to each well. Samples were mixed by pipetting 6 times and shaking for 5 min at room temperature. Magnetic separation was achieved by placing the block on a NucleoMag SEP magnetic separator for 2 min, after which the supernatant was discarded. The beads were washed with 600 µL wash buffer (MB3) per well and shaken for 5 min to resuspend the beads, followed by another 2-min magnetic separation and removal of the supernatant. This washing step was repeated using wash buffer MB4. After a further 2-min separation, 900 µL wash buffer MB5 was added and incubated for 45 to 60 s while still on the magnetic separator, after which the supernatant was discarded. Following removal of the block from the magnetic separator, 50 to 200 µL elution buffer (MB6) was added and mixed by shaking for 5 to 10 min at 56°C. In the final step, magnetic separation was performed once more for 2 min, and the supernatant

containing purified DNA was transferred to a suitable collection tube.

Genotyping was performed using the Infinium HTS Assay (Illumina). First, 4 μ L of purified DNA was mixed with 20 μ L Multi-Sample Amplification Mix 1 and 4 μ L of 0.1 N sodium hydroxide (NaOH) in the appropriate wells of a midi plate. After sealing, the plate was vortexed at 1,600 rpm for 1 min and pulse-centrifuged at $280 \times g$ at room temperature using a Rotina 420R centrifuge (Hettich, Tuttlingen, Germany). Following a 10-min incubation at room temperature, 34 μ L Multi-Sample Amplification Mix 2 and 38 μ L Multi-Sample Amplification Master Mix were added to each well. The plate was resealed, vortexed, pulse-centrifuged, and incubated in an Illumina Hybridization Oven for 20 to 24 h at 37°C. On the following day, after pulse centrifugation at $50 \times g$ at room temperature for approximately 5 s, 25 μ L fragmentation solution was added to each well. After vortexing and centrifugation, the plate was incubated on a preheated heat block for 1 h at 37°C. Subsequently, 50 μ L precipitation solution was added, followed by vortexing and incubation for 5 min at 37°C. After pulse centrifugation at $280 \times g$ for 1 min at room temperature, 155 μ L 2-propanol was added, and the plate was sealed with a new cap mat. After inversion of the plate 10 times, the plate was incubated at 4°C for 30 min and then centrifuged at $3,000 \times g$ for 20 min at room temperature. The plate was immediately inverted over an absorbent pad to remove the supernatant, left inverted for ~1 min, and air-dried at room temperature for 1 h. The precipitated DNA was resuspended in 23 μ L resuspension, hybridization, and wash solution (RA1) and incubated for 1 h at 48°C. After vortexing and pulse centrifugation, the DNA fragments were prepared for hybridization on BeadChips. The plate was heated to 95°C for 20 min to denature the DNA, then cooled at room temperature for 30 min. After pulse centrifugation, 14 μ L of each sample was applied to the appropriate section of a preprepared BeadChip with 400 μ L humidifying buffer. BeadChips were placed in hybridization chambers and incubated at 48°C for 24 h. Following hybridization, BeadChips were washed twice for 1 min each in wash proteinase buffer (PB1). They were then placed in flow-through chambers maintained at 44°C and extended with labeled nucleotides using a series of incubation steps with RA1, Long XStain solutions, Extension Mix Long, and EDTA, according to the manufacturer's protocol. Staining was performed using Superior Two-Color Master Mix and Anti-Stain Two-Color Master Mix, with intermediate washing steps using XC3. After final staining and washing, BeadChips were placed in PB1 at room temperature, washed by gentle mixing, and subsequently washed in XC4. BeadChips were then dried in a vacuum desiccator for 50 min at

675 mmHg and scanned using the Infinium LCG. Each BeadChip contains information on ~50,000 SNPs. The SNP data were processed using the Illumina Genocells statistical model, which calculated the individual SCC values of all cows using the following formula (Lenormand et al., 2019):

$$(\% \text{ DNA of each cow} / \% \text{ milk of each cow} \\ \text{in the bulk tank}) \times \text{Bulk tank cell count.}$$

A detailed presentation of the calculation can be found in the publication by Blard et al. (2012).

Individual milk samples for MR were collected during milking directly from the milk collection unit into 40-mL containers containing 150 μ L liquid Bronysolv for preservation. Samples were transported to the laboratory of Milchprüfing Bayern within 1 to 2 d and analyzed using the Bentley FTS/FCM 500 combi system, which combines fluorescence cytometry for SCC determination with Fourier-transform spectroscopy for milk component analysis (Bentley Instruments, Inc.). All procedures were carried out according to the manufacturer's instructions.

Statistical analyses and graphical illustrations were performed using R statistical software (version 4.4.0; Posit Team, 2025). To minimize the influence of outliers and because the SCC results of a herd are gamma distributed, SCC values were logarithmized using the natural logarithm [$\ln(\text{SCC})$]. Due to repeated measurements, a linear mixed-effects model was used for analysis of $\ln(\text{SCC})$, with sampling included as a fixed effect and cow identity as a random effect. For the comparison of the 10 different samples to each other, separate *P*-values for every 2 samples against each other were calculated. Comparisons between measurement methods were conducted using Spearman correlation analysis because the data were not normally distributed. The lowest value both measuring methods can display is 10,000 cells/mL, even if no cells at all were detected in a sample. That is why we decided to exclude all 246 results with a result of 10,000 cells/mL in 1 of the 2 methods from the analysis. For further evaluation, results were categorized into 4 SCC classes: <100,000 cells/mL, 101,000 to 200,000 cells/mL, 201,000 to 400,000 cells/mL, and >400,000 cells/mL. Data pairs were assigned to these classes and analyzed for deviations exceeding one SCC class. Finally, SCC classes were compared using a linear mixed-effects model with measurement method as a fixed effect and cow as a random effect. A *P*-value of ≤ 0.05 was considered significant. Because no treatment was performed following the results, no blinding of the persons analyzing the data was necessary.

RESULTS

Figure 1 shows the results of the reproducibility trial of the Genocells method. Ten bulk milk samples collected on the same day were analyzed separately, and the SCC results are displayed for each cow in the herd as individual boxplots. Ninety per cent of cows showed an SD of less than 100,000 cells/mL across the 10 samples. Of the remaining 6 cows, 3 showed an SD below 200,000 cells/mL, and only 1 cow showed an SD exceeding 400,000 cells/mL.

In Figure 2, the ln-transformed SCC values [$\ln(\text{SCC})$] from the 10 samples of the same bulk milk tank were compared with one another. The results of the different samples showed no significant differences between each other (F comparison of P -values between all samples revealed no significant differences, in fact, all of the 45 calculated P -values were bigger than 0.12, and 37 calculated as 1.00).

To assess the reliability of the Genocells method, results were compared with SCC values obtained by FC from individual milk samples collected on the same day using Spearman correlation analysis. The correlation between the 2 measurement methods was highly significant ($P < 0.001$), with a correlation coefficient (r) of 0.83 (Figure 3).

In the next step, SCC results were categorized into 4 classes (<100,000 cells/mL, 101,000–200,000 cells/mL,

201,000–400,000 cells/mL, and >400,000 cells/mL). The frequency with which both methods assigned results to the same SCC class was analyzed. Where classifications differed, both the frequency and magnitude of disagreement were assessed (Figure 4).

Overall, 81.5% of SCC results obtained on the same day were assigned to the same SCC class by both measurement methods. A deviation of more than one SCC class was observed in only 1.5% of all cases (Figure 4).

Next, a linear mixed-effects model of the ln-transformed SCC was used to assess whether significant differences between the 2 measurement methods were present within individual SCC classes. Cow identity was included as a random effect, while measurement method was included as a fixed effect.

A significant difference between methods was observed only for SCC values below 100,000 cells/mL (Figure 5). In the SCC classes 101,000 to 200,000 cells/mL, 201,000 to 400,000 cells/mL, and >400,000 cells/mL, no significant differences between the measurement methods were detected.

DISCUSSION

The aim of this study was to evaluate whether the Genocells method (an approach that estimates individual SCC in a bulk tank milk sample using SNP-based cow

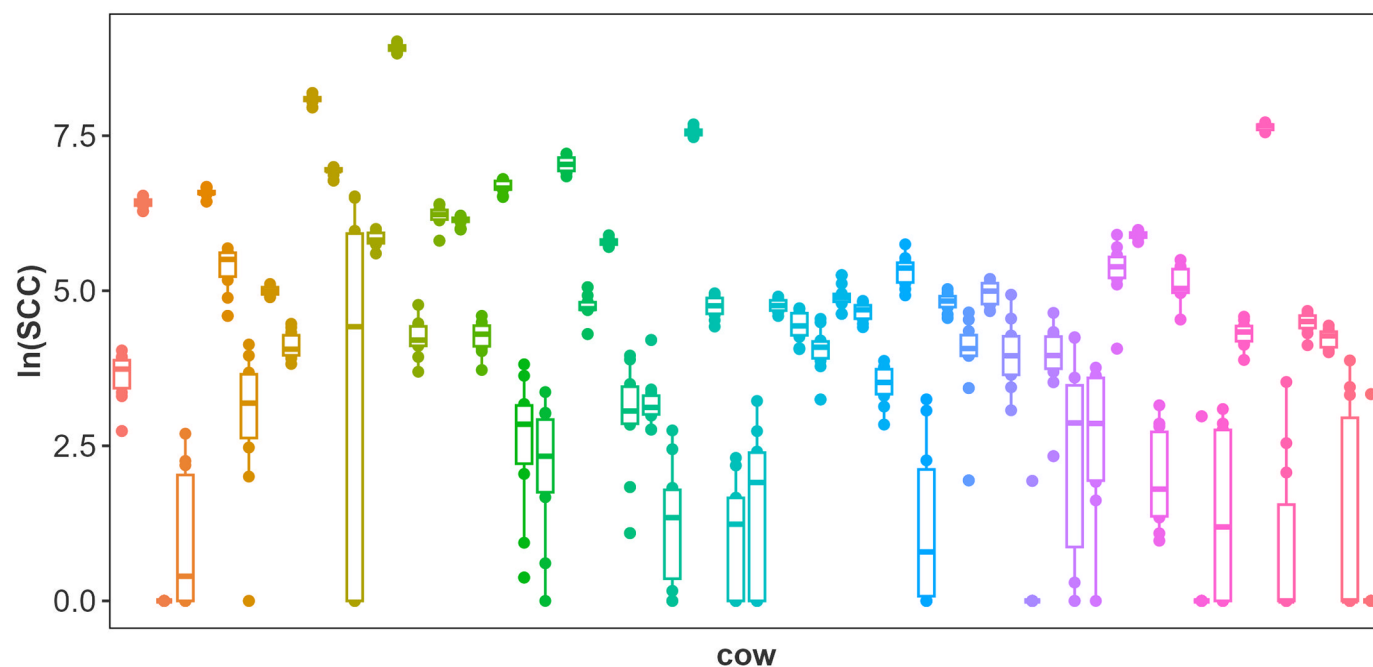


Figure 1. Individual variation of SCC in 10 milk samples from the same day. All 60 cows of the herd are displayed on the x-axis. The y-axis shows the ln-transformed SCC derived from bulk milk analysis using the Genocells method. Ten bulk milk samples collected on the same day were analyzed, and results are presented as one boxplot per cow, each composing 10 SCC measurements. The box symbolizes the interquartile range (IQR) containing 75% of the results. The solid line in the box shows the median. The whiskers show the distance to the lowest and highest result, that is still within $1.5 \times$ the IQR. The single dots show the outliers.

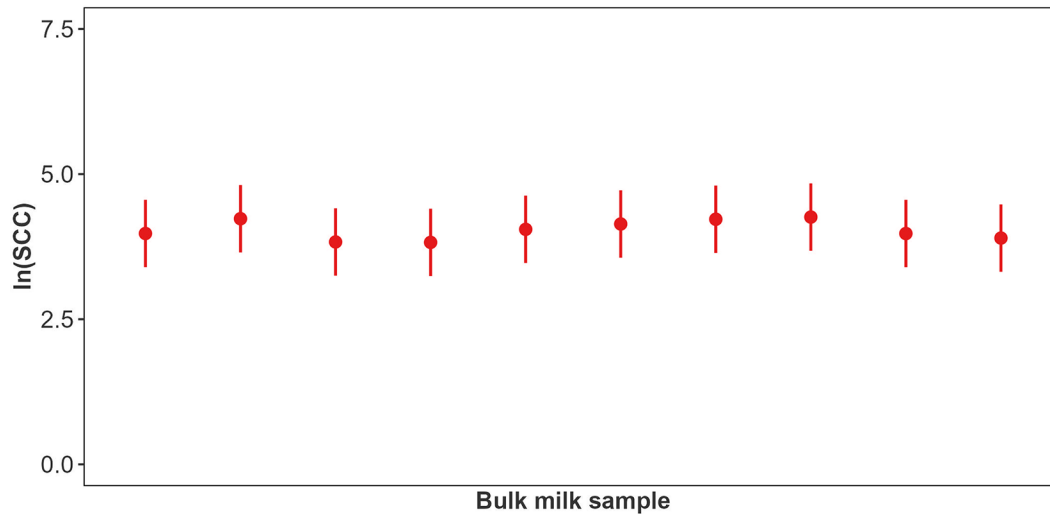


Figure 2. Variation between same-day bulk milk samples. The individual SCC of all 60 cows in one herd was determined from 10 different samples taken from the same bulk milk using the Genocells method. Samples are shown on the x-axis, whereas individual SCC values are displayed on the y-axis and have been ln-transformed for improved comparability. The red circles show the median. The lines show the range of the results. *P*-values were calculated using a mixed-effects model with a significance level of $P < 0.05$. No significant differences were observed between any of the samples.

identification) can serve as a reliable alternative to conventional FC performed on individual milk samples.

The SCC remains the most widely used indicator for assessing udder health at both cow and herd level. The SCC primarily reflects the immunological status of the

cow, but it is also influenced by environmental and physiological factors such as stress, weather, and stage of lactation. Daily variation in SCC has been estimated at 2.0% to 4.7% in healthy cows and 4.0% to 11.4% in subclinically or clinically infected cows (Chagunda et

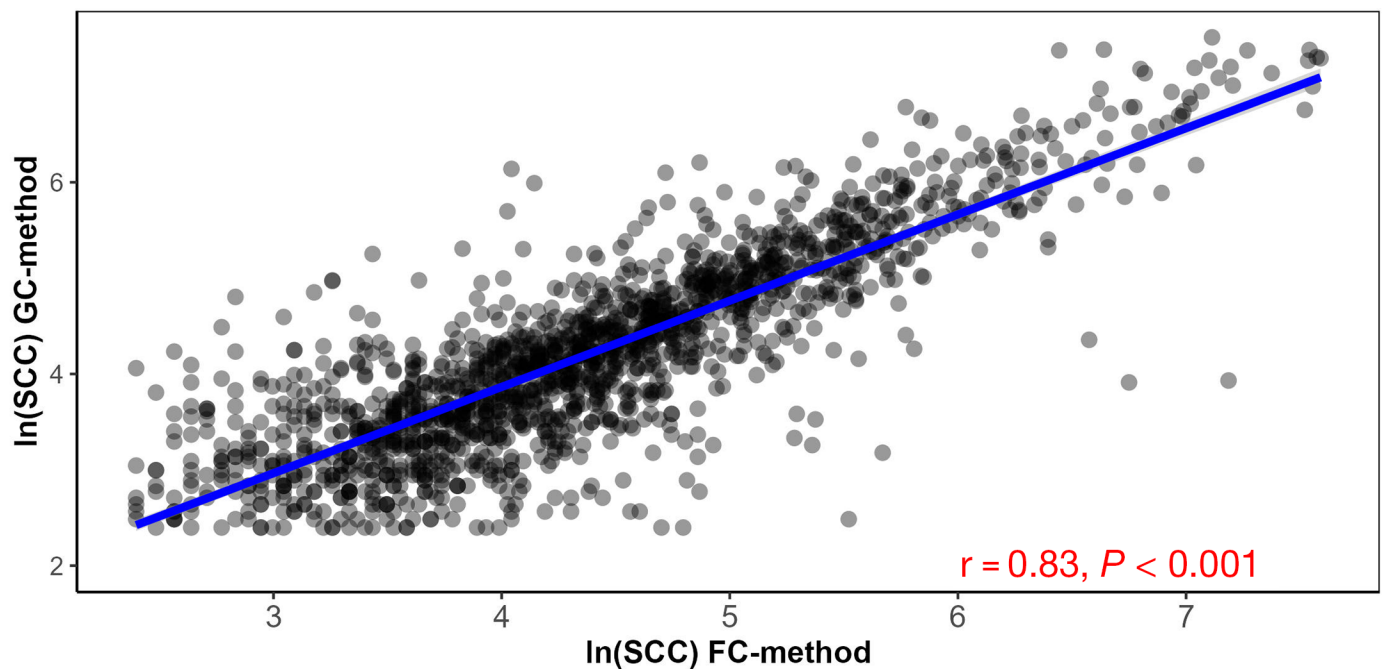


Figure 3. Correlation between flow cytometry (FC) and Genocells results obtained on the same day. Shown is the Spearman correlation of logarithmized SCC results obtained using 2 different measurement methods. Each point represents the SCC of one cow measured by FC (x-axis) and Genocells (y-axis) on the same day. The correlation coefficient and *P*-value are indicated in red.

Genocell method	Flow cytometry method			
	<100,000/mL	101,000/mL– 200,000/mL	201,000/mL– 400,000/mL	>400,000/mL
<100,000/mL	1,198 (63.7%)	139 (7.4%)	11 (0.6%)	4 (0.2%)
101,000/mL–200,000/mL	51 (2.7%)	178 (9.4%)	54 (2.9%)	1 (0.1%)
201,000/mL–400,000/mL	7 (0.3%)	33 (1.7%)	93 (4.9%)	26 (1.4%)
<400,000/mL	1 (0.1%)	4 (0.2%)	16 (0.9%)	66 (3.5%)

Figure 4. Consistency of SCC category assignment between measurement methods. Green cells indicate agreement between methods (same SCC category). Yellow cells indicate a deviation of one SCC category between methods. Red cells indicate a deviation of more than one SCC category. Overall percentages are displayed.

al., 2006; Forsbäck et al., 2010). Consequently, a method that enables frequent, labor-efficient, and cost-effective data collection is of considerable value for udder health surveillance.

The FC, commonly used in monthly MR, yields slightly higher SCC values than the gold standard of light microscopy but shows strong correlation with it ($r = 0.90\text{--}0.99$; Feng and Zheng, 2005; Zajac et al., 2016). However, FC requires individual cow samples and is therefore labor-intensive and costly (~€2.50 per sample; Bayern, 2025). As a result, cows are typically tested only 11 times per year. On-farm tools such as the DeLaval cell counter (DCC) allow more frequent measurements and show high agreement with FC, particularly below 200,000 cells/mL, where 98% of results correlate (Enger et al., 2020). Limitations include reduced accuracy at higher SCC levels, a detection limit of 4 million cells/mL for the DCC (Hisira et al., 2023), and the continued requirement for individual cow sampling, which restricts applicability for routine whole-herd monitoring.

The SNP-based individual identification from a single bulk tank milk sample may therefore represent a labor- and cost-saving alternative.

The intra-assay comparison of 10 bulk tank samples collected from the same tank on the same day demonstrated high reproducibility of the Genocells method. For 90% of cows, variation among the 10 Genocells measurements was within 100,000 cells/mL, and only 1 cow exceeded a deviation of 400,000 cells/mL. Mixed-effects model analysis revealed no significant differences between samples, indicating minimal technical variation in genotyping and limited sampling-related variance. These findings highlight the robustness of Genocells-based SCC estimation, which is a critical requirement for diagnostic methods relying on bulk tank samples. In the study by Enger et al. (2020), all samples were measured twice, with mean within-sample variation reported as 28% for light microscopy, 12% for DCC, and 6% for FC.

Because previous studies have shown that FC results are comparable to light microscopy (Feng and Zheng,

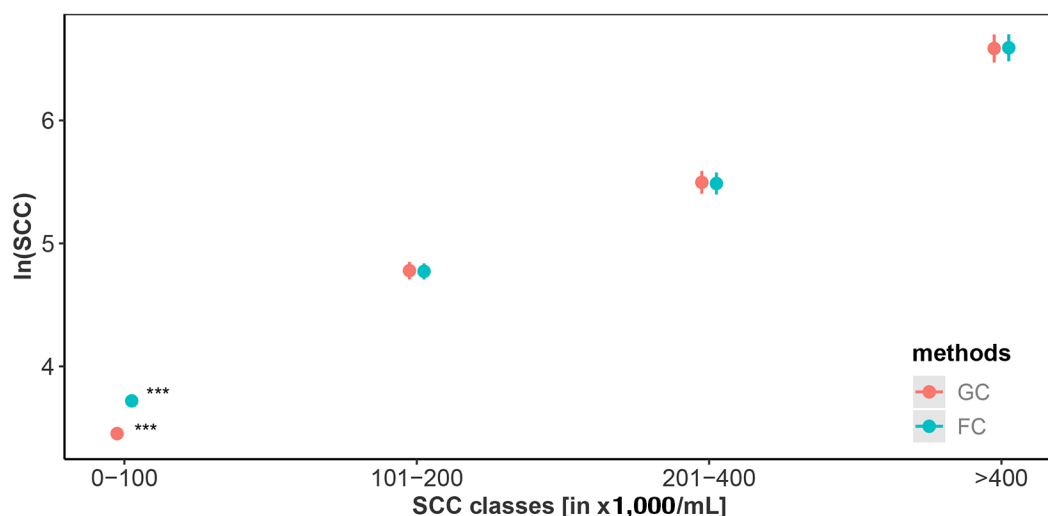


Figure 5. Comparison of SCC results between measurement methods across SCC classes. Individual SCC results obtained on the same day using 2 different measurement methods were analyzed. The SCC from individual milk samples was determined by FC (flow cytometry), whereas SCC from bulk milk samples collected on the same day was determined using the Genocells method. The SCC classes are shown on the x-axis, and ln-transformed SCC values are shown on the y-axis. P -values were calculated using a mixed-effects model with a significance level of $P < 0.001$. ***A significant difference between methods was observed only in the SCC class below 100,000 cells/mL.

2005; Hisira et al., 2023), this widely used method was selected as the benchmark for comparison with the Genocells approach. The present study identified a strong correlation between Genocells and FC results ($r = 0.83$; $P < 0.001$), which is comparable to reported agreements between FC and other SCC testing methods (Gonzalo et al., 2003; Hisira et al., 2023). This indicates that Genocells provides SCC estimates consistent with established diagnostic standards.

When SCC results were categorized into defined classes, 81.5% of paired Genocells–FC values fell within the same category, and only 1.5% differed by more than one category. Because udder health decisions are often based on SCC thresholds rather than exact numerical values, this high level of agreement is particularly relevant for clinical decision-making and herd management.

A significant difference between methods was observed only for SCC values below 100,000 cells/mL, likely reflecting the relatively small contribution of low-SCC cows to the bulk tank sample. This increases susceptibility to proportional errors or minor technical variation. Comparable limitations have been reported for other low-range SCC tests, such as the California Mastitis Test and certain on-farm counters. Importantly, this limitation did not impair detection of clinically relevant or at-risk cows.

A major advantage of the Genocells approach is the substantial reduction in sampling effort. Whereas conventional SCC monitoring requires individual milk samples from each cow, the Genocells method relies on a single bulk tank milk sample to estimate individual SCC values. This markedly reduces labor, logistical complexity, and overall costs. The approach is therefore particularly suitable for increasing sampling frequency, which supports early detection of rising SCC values. Shorter monitoring intervals are known to be critical for mastitis control because early treatment improves cure rates and reduces milk losses. In particular, cows with SCC values between 100,000 and 200,000 cells/mL warrant special attention because they are likely to represent recent infections with the highest potential for successful early treatment (Torres et al., 2008).

Monthly SCC testing limits the ability to distinguish newly acquired infections from older infections or transient SCC elevations related to stress (Nasr and El-Tarabany, 2017; Hong et al., 2019; Moore et al., 2023). Increased sampling frequency, made feasible by Genocells, improves interpretation of SCC dynamics and enhances detection of true intramammary infections.

Because Genocells relies on SNP-based genotyping, it is inherently robust to sample mix-ups, which are a known source of error in routine MR. However, the method requires all cows in the herd to be genotyped, which may limit adoption in regions where genotyping

is not yet routine practice. Genotyping can be performed using skin samples collected during ear tagging and typically costs between €25 and €45 per cow, with reduced costs available to farms participating in breeding programs such as KuhVision (<https://www.ruweg.de/zucht/kuhvision>). Because genetics play an increasingly important role in predicting future milk yield and performance, a growing number of farmers already use genotyping to inform selection decisions (Stöcker, 2017). A technical limitation in this study was the sample transport time (2–6 d), which likely contributed to the exclusion of samples with pH values below 6.40. Faster logistics or decentralized sample processing could further improve accuracy and reduce sample loss. Because of the sampling protocol, the results of one individual milk sample might have been compared with one or 2 milking times in a bulk milk sample, depending on when the bulk tank was emptied. The results are still correlated strongly, and the fact that the Genocells results are an average of potentially more than one milking also might show a more realistic picture that is less influenced by one-time effects. In addition, because of the limited number and characteristics of participating farms, breed-specific effects and the influence of different milking systems could not be evaluated.

The Genocells method performed reliably in herds of 62 to 85 cows. Previous work suggests that to maintain a good SCC determination, the chip size may need to be adjusted according to herd size (Blard et al., 2012). Blard et al. (2012) showed that for about 100 individuals a 50K chip is enough. Future studies should therefore assess Genocells performance in larger herds and in automated milking systems, where milking frequency and milk fractions differ from conventional parlors.

Further research is also needed to determine how effectively Genocells can capture SCC dynamics over shorter time intervals. Reported daily SCC fluctuations range from 2% to 8%, and some cows exhibit cyclic SCC patterns over several days (Forsbäck et al., 2010; Deng et al., 2021). A method that enables frequent, low-effort sampling may help distinguish true mastitis cases from physiological variation more effectively than current monthly testing regimens.

In addition, future studies should examine whether genetically identical animals, such as monozygotic twins, can be reliably differentiated using the current SNP panel.

CONCLUSIONS

Within the framework of this study, the Genocells method was implemented in Germany for the first time. Following its development at the University of Liège, Belgium, the method has been used commercially in France since 2018; however, no published data on its

performance under practical conditions were available before this study. The findings of this study support the Genocells method as a reliable tool for estimating individual SCC from a single bulk tank milk sample. The method demonstrated high reproducibility, strong agreement with established FC, and accurate classification across clinically relevant SCC thresholds. By substantially reducing sampling effort, Genocells has the potential to improve herd-level udder health monitoring, enable more frequent SCC assessment, and support earlier detection of mastitis in individual cows using only a single bulk milk sample. Future research should focus on defining the maximum practical herd size, optimizing sample logistics, evaluating performance across diverse production systems, and exploring predictive modeling approaches that integrate Genocells data with mastitis records to further enhance early detection of SCC changes in individual cows.

NOTES

This work is part of the IQexpert project. The project was supported by funds from the Federal Ministry of Food and Agriculture (Bonn, Germany; BMEL), based on a decision of the Parliament of the Federal Republic of Germany, and administered via the Federal Office for Agriculture and Food (Bonn, Germany; BLE) under the Innovation Support Program. The data were provided by the Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. The authors used an official proof-reading service and ChatGPT (OpenAI) to assist with language revision of the discussion. All scientific content and interpretations remain the responsibility of the authors. No human or animal subjects were used; therefore, this analysis did not require approval by an Institutional Animal Care and Use Committee or an Institutional Review Board. One of the authors is an employee of the company that commercializes the measurement method evaluated in this study. This author was not involved in analyzing the results, therefore, the authors consider the potential conflict of interest as negligible. The authors have not stated any other conflicts of interest.

Nonstandard abbreviations used: DCC = DeLaval cell counter; FC = flow cytometry; IQR = interquartile range; MR = milk recording; PB1 = proteinase buffer; RA1 = resuspension, hybridization, and wash solution; T1 = lysis buffer.

REFERENCES

- Barbosa, L. F. S. P., W. V. C. Oliveira, M. H. C. Pereira, M. B. Moreira, C. G. C. Vasconcelos, B. F. Silper, R. L. A. Cerri, and J. L. M. Vasconcelos. 2018. Somatic cell count and type of intramammary infection impacts fertility from in vitro produced embryo transfer. *The-riogenology* 108:291–296. <https://doi.org/10.1016/j.theriogenology.2017.12.025>.
- Blard, G., Z. Zhang, W. Coppieters, and M. Georges. 2012. Identifying cows with subclinical mastitis by bulk single nucleotide polymorphism genotyping of tank milk. *J. Dairy Sci.* 95:4109–4113. <https://doi.org/10.3168/jds.2011-5178>.
- Chagunda, M. G., T. Larsen, M. Bjerring, and K. L. Ingvarsen. 2006. L-lactate dehydrogenase and N-acetyl-beta-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. *J. Dairy Res.* 73:431–440. <https://doi.org/10.1017/S0022029906001956>.
- Chen, H., A. Weersink, D. Kelton, and M. von Massow. 2021. Estimating milk loss based on somatic cell count at the cow and herd level. *J. Dairy Sci.* 104:7919–7931. <https://doi.org/10.3168/jds.2020-18517>.
- Cinar, M., U. Serbester, A. Ceyhan, and M. Gorgulu. 2015. Effect of somatic cell count on milk yield and composition of first and second lactation dairy cows. *Ital. J. Anim. Sci.* 14:3646. <https://doi.org/10.4081/ijas.2015.3646>.
- Deng, Z., T. J. G. M. Lam, H. Hogeveen, and G. Koop. 2021. Regularly fluctuating somatic cell count pattern in dairy herds. *J. Dairy Sci.* 104:11126–11134. <https://doi.org/10.3168/jds.2020-20063>.
- Enger, B. D., S. C. Nickerson, R. M. Akers, L. E. Moraes, and C. E. Crutchfield. 2020. Use of commercial somatic cell counters to quantify somatic cells in non-lactating bovine mammary gland secretions. *Prev. Vet. Med.* 174:104775. <https://doi.org/10.1016/j.prevetmed.2019.104775>.
- Feng, W., and X. Zheng. 2005. Comparing techniques for detecting the number of somatic cells in raw milk. *Eur. Food Res. Technol.* 220:653–657. <https://doi.org/10.1007/s00217-004-1084-1>.
- Forsbäck, L., H. Lindmark-Månsson, A. Andrén, M. Åkerstedt, L. Andrén, and K. Svennersten-Sjaunja. 2010. Day-to-day variation in milk yield and milk composition at the udder-quarter level. *J. Dairy Sci.* 93:3569–3577. <https://doi.org/10.3168/jds.2009-3015>.
- Gonzalo, C., J. R. Martínez, J. A. Carriedo, and F. San Primitivo. 2003. Fossomatic cell-counting on ewe milk: Comparison with direct microscopy and study of variation factors. *J. Dairy Sci.* 86:138–145. [https://doi.org/10.3168/jds.S0022-0302\(03\)73593-0](https://doi.org/10.3168/jds.S0022-0302(03)73593-0).
- Hand, K. J., A. Godkin, and D. F. Kelton. 2012. Milk production and somatic cell counts: A cow-level analysis. *J. Dairy Sci.* 95:1358–1362. <https://doi.org/10.3168/jds.2011-4927>.
- Hisira, V., F. Zigo, M. Kadaši, R. Klein, Z. Farkašová, M. Vargová, and P. Mudroň. 2023. Comparative analysis of methods for somatic cell counting in cow's milk and relationship between somatic cell count and occurrence of intramammary bacteria. *Vet. Sci.* 10:468. <https://doi.org/10.3390/vetsci10070468>.
- Hong, H., E. Lee, I. H. Lee, and S.-R. Lee. 2019. Effects of transport stress on physiological responses and milk production in lactating dairy cows. *Asian-Australas. J. Anim. Sci.* 32:442–451. <https://doi.org/10.5713/ajas.18.0108>.
- Huang, C.-H., and N. Kusaba. 2022. Association between differential somatic cell count and California Mastitis Test results in Holstein cattle. *JDS Commun.* 3:441–445. <https://doi.org/10.3168/jdsc.2022-0249>.
- Kortstegge, J., K. Persson Waller, C. G. M. Scherpenzeel, J. Starič, L. Pinho, P. Kovacs, O. Salat, A. M. Biggs, P. Moroni, and V. Krömker. 2024. Different European perspectives on the treatment of subclinical mastitis in dairy cows. *MSI* 77. <https://doi.org/10.48435/MSI.2024.2>.
- Lenormand, P., F. Perrin, M. Calmels, V. Charrier, M. Foucher, and J. B. Davière. 2019. GenoCells: Individual somatic cell count of dairy cows by genotyping tank milk. ICAR Technical Series No. 24. Accessed Jun. 18, 2026. https://www.icar.org/Documents/technical_series/ICAR-Technical-Series-no-24-Prague/Lenormand.pdf.
- LKV Bayern. 2025. Beitragsordnung. Accessed Dec. 14, 2025. <https://www.lkv.bayern.de/formulare-und-preise/>.
- Löf, E., H. Gustafsson, and U. Emanuelson. 2014. Factors influencing the chance of cows being pregnant 30 days after the herd voluntary waiting period. *J. Dairy Sci.* 97:2071–2080. <https://doi.org/10.3168/jds.2012-5874>.
- Moore, S. S., A. Costa, M. Penasa, S. Callegaro, and M. de Marchi. 2023. How heat stress conditions affect milk yield, composition, and price in Italian Holstein herds. *J. Dairy Sci.* 106:4042–4058. <https://doi.org/10.3168/jds.2022-22640>.

- Nasr, M. A. F., and M. S. El-Tarabany. 2017. Impact of three THI levels on somatic cell count, milk yield and composition of multiparous Holstein cows in a subtropical region. *J. Therm. Biol.* 64:73–77. <https://doi.org/10.1016/j.jtherbio.2017.01.004>.
- Pinedo, P. J., P. Melendez, J. A. Villagomez-Cortes, and C. A. Risco. 2009. Effect of high somatic cell counts on reproductive performance of Chilean dairy cattle. *J. Dairy Sci.* 92:1575–1580. <https://doi.org/10.3168/jds.2008-1783>.
- Posit Team. 2025. RStudio: Integrated development environment for R. Posit Software, PBC, Boston, MA. Accessed Jun. 17, 2026, <http://www.posit.co/>.
- Redelman, D., S. Butler, J. Robison, and D. Garner. 1988. Identification of inflammatory cells in bovine milk by flow cytometry. *Cytometry* 9:463–468. <https://doi.org/10.1002/cyto.990090510>.
- Rivas, A. L., F. W. Quimby, J. Blue, and O. Coksaygan. 2001. Longitudinal evaluation of bovine mammary gland health status by somatic cell counting, flow cytometry, and cytology. *J. Vet. Diagn. Invest.* 13:399–407. <https://doi.org/10.1177/104063870101300506>.
- Stöcker, C. 2017. Weibliche Nachzucht genotypisieren - mehr als nur Kosten? *Elite Magazin*, Oct. 19, 2017. Accessed Dec. 14, 2025. <https://www.elite-magazin.de/news/nachrichten/weibliche-nachzucht-genotypisieren-mehr-als-nur-kosten-11625.html>.
- Sun, X., R. Zhao, N. Wang, J. Zhang, B. Xiao, F. Huang, and A. Chen. 2023. Milk somatic cell count: From conventional microscope method to new biosensor-based method. *Trends Food Sci. Technol.* 135:102–114. <https://doi.org/10.1016/j.tifs.2023.03.020>.
- Torres, A. H., P. J. Rajala-Schultz, F. J. Degraives, and K. H. Hoblet. 2008. Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. *J. Dairy Res.* 75:240–247. <https://doi.org/10.1017/S0022029908003257>.
- Zajac, P., S. Zubricka, J. Capla, and L. Zelenakova. 2016. Fluorescence microscopy methods for the determination of somatic cell count in raw cow's milk. *Vet. Med. (Praha)* 61:612–622. <https://doi.org/10.17221/222/2015-VETMED>.

ORCIDS

- A. Pichlmeier, <https://orcid.org/0009-0008-5613-3407>
 L. Ziegler, <https://orcid.org/0009-0009-9120-3949>
 Y. Zablotzki, <https://orcid.org/0000-0001-6928-4089>
 M. Iwersen, <https://orcid.org/0000-0001-7893-6050>
 H. Zerbe, <https://orcid.org/0000-0002-7972-4858>
 R. Martin <https://orcid.org/0009-0008-1660-0373>