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# MEASUREMENT OF MICROBIOLOGICAL QUALITY OF RAW GOAT´S MILK BY LASER FLOW CYTOMETRY

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**ABSTRACT**

The popularity of goat's milk and its products is growing in Slovakia. Among the mandatory health safety features of raw goat's milk is the total bacterial count. It can be determined by the anchor plate-cultivation method, which is, however, tedious, and laborious. Laser flow cytometry is a modern alternative method for measuring the microbiological quality of raw milk. To use the results from laser flow cytometry method for legislative purposes, it is necessary to create a representative conversion of the primarily measured results obtained by this method into the CFU/mL scale. This work describes the creation of such a conversion for raw goat's milk, which will be valid for the Slovak region. After measuring several hundred individual and bulk tank samples of raw goat's milk on a BactoScan FC laser flow cytometer and at the same time using the anchor method according to STN EN ISO 4833-1 and after their subsequent logarithmic transformation, such a conversion was created in the form of $\log_{10}$(CFU/mL) = 1.0174 x $\log_{10}$(IBC/µl) + 2.7483. Subsequently, this was verified by comparing the recalculated and directly measured results in CFU/mL, using additional data set of samples. The influence of the somatic cell count in raw goat's milk, as a possible interfering factor, was also assessed.

**Keywords:** laser flow cytometry, total bacterial count, raw goat´s milk, somatic cell count

## INTRODUCTION

The total bacterial count and the residues of antibiotics are the basic and mandatory indicators of the quality of milk from species other than cows, according to European food law **(Regulation (EC) No 853/2004)**. The anchor/reference method for measuring the total bacterial count is the plate cultivation method according to **STN EN ISO 4833-1**. The disadvantage of this method is that it is laborious and tedious - the results are available in as little as three days. Therefore, routine, and fast methods can be used to measure this parameter. However, a mandatory condition **(Commission Regulation (EC) No 2074/2005, Commission Regulation (EC) No 1664/2006)** is that these methods have been validated against the reference method in accordance with the requirements of **ISO 16140-2**.
Laser flow cytometry is a modern method that has been used for a long time in microbiology, biology, and medicine **(Robinson, 2022)**. In the field of dairy microbiology, it began to be used intensively after the introduction of single-purpose laser flow cytometers, specifically for measuring the total bacterial count. The advantage of these cytometers is that the measurement is automated and fast – the result is measured within a few minutes. Thus, the farmer or dairy can immediately implement possible corrective measures if the hygienic quality of the milk is poor. The disadvantage of this method is that it does not primarily measure the results in CFU/mL units, in which the legislative limits for acceptable milk quality are stated. In addition, for each of the manufacturers of laser flow cytometers, this measurement is slightly different, and thus such results cannot be always exactly compared with each other. Thus, in most countries where such cytometers are routinely used, directly measured results are mostly converted to the CFU/mL scale. The created calculations are either at the national or regional level and may differ from each other **(Bulletin International Dairy Federation 511/2021)**.
A typical mathematical method for creating a conversion equation for directly measured results from flow cytometers to the CFU/mL scale is to construct a linear regression equation after logarithmic transformation of the results. Directly measured results are displayed on the x-axis and results measured by the plate method on the y-axis. When analysing the data, it is necessary to verify that the measurement is linear throughout the measurement range **(Suhren and Reichmuth, 2000)**. To achieve the conversion relationship to be reliable, it is necessary to create a representative data set of results from both methods. The samples used for this must represent the geographical region where the conversion will be applied, considering the type of milk tested, the breeds of animals, the method of rearing and the size of herds, the method of rearing and nutrition, the method used for milking, sampling, and preservation of samples, as well as handling them until the time of testing **(ISO 21187).**

Measurement of the total bacterial count using laser flow cytometry in raw cow's milk was introduced in Slovakia in 2004 **(Tomáška and Suhren, 2004)** and in raw sheep's milk in 2006 **(Tomáška *et al.*, 2006)**. Based on the created conversions, it was found that these differ according to the type of milk tested. For greater reliability of the measurement, it is therefore advisable to have a representative conversion made especially for cow's, sheep's, and goat's milk. This statement is already justified in the fact that, for example, raw sheep's and goat's milk mostly contain higher numbers of microorganisms than raw cow's milk. In addition, in raw sheep's and goat's milk, the number of somatic cells present is higher **(Paape *et al.*, 2007)**, while in addition, in raw goat's milk, the somatic cells are smaller and thus can interfere with the actual measurement of microorganisms on laser flow cytometers.
Experience with measuring the microbiological quality of raw goat's milk using laser flow cytometry is described by **Suhren *et al.* (2005)** and **Ramsahoi *et al.* (2011)**. The application of this knowledge, as well as the previous experience of the authors **(Tomáška *et al.*, 2023a)**, were used to introduce the laser flow cytometry method and to create a representative calculation of the results for the raw goat's milk matrix in Slovakia.

## MATERIAL AND METHODS

### Samples

Samples of raw goat's milk, used to measure the total bacterial count by the plate-cultivation method and the method based on the principle of laser flow cytometry, the residues of antibiotics and the somatic cell count were measured in the accredited testing laboratory EXAMINALA, Výskumný ústav mliekárenský, a.s. Žilina in the period of 2021 and 2023. These were bulk tank samples as well as individual samples.
Samples were taken every month from 21 goat milk breeders and processors from all over Slovakia. The milk came from different breeds of goats that were raised on pasture, in a barn or combined. The size of herds varied from individual animals to several dozen animals. Milk was milked by hand or by machine.
Samples after milking were preserved with Acidiol and kept at temperatures up to 8°C until the time of testing (usually within 48 hours after collection). First, the samples were tested for the residues of antibiotics, then the total bacterial count and then the somatic cell count.

**Methods**

The residues of inhibitory of antibiotics were tested by Delvotest SP NT kit (DSM Food Specialties B.V, Delft, The Netherlands) according to procedure by manufacturer.
The total bacterial count was measured by the anchor plate-cultivation method according to **STN EN ISO 4833-1** and by laser flow cytometry method using BactoScan FC analyser (FOSS, Hillerød, Denmark), equipped with FOSS Integrator software.
The somatic cell count was measured by the method according to **STN EN ISO 13366** using Fossomatic 7 analyser (FOSS, Hillerød, Denmark), equipped with FOSS Integrator software.
The quality of testing was achieved by introduction of regular control tools, like the calibration of equipment (Fossomatic, the calibration samples for somatic cells in goat milk, the measurement of the reference materials, evaluation of repeatability, carry-over, blank-check, and participation in interlaboratory studies. All calculations and statistic evaluation were done by MS Excel (Microsoft, Richmond, USA).

**RESULTS AND DISCUSSION**

As mentioned in the introduction, to establish a reliable conversion of the microbiological quality results measured by laser flow cytometry into CFU/mL scale, it is crucial to create a representative set of milk samples from which the results will be calculated. Raw goat´s milk samples tested in 2021 and 2022 were used to create the conversion. Samples taken in 2023 were used to verify it.
All samples used in the study do not contain residues of antibiotics.

**Quality of raw goat's milk in Slovakia**

Table 1 shows a basic overview of the microbiological quality of raw goat's milk in Slovakia in the period 2021-2023. The conclusions published for the period 2021-2022 **(Tomáška *et al.*, 2023b)** were confirmed and that the microbiological quality of raw goat milk is good. Up to 79.5% of the samples had a total bacterial count value lower than 500,000 CFU/mL, which is the limit set by legislation **(Regulation (EC) No 853/2004)** for raw goat's milk, which does not need to be further heat treated. For milk that is heat-treated, the same legislation sets a limit at the level of 1,500,000 CFU/mL, while up to 89.9% of samples met this limit. The geometric mean of all tested samples was at the level of 54,000 KTJ/ml, the arithmetic mean was 270,000 CFU/mL. It is clear from these data that although the samples of raw goat's milk in most cases complied with the legislative limits, the average of the total bacterial count values achieved in this milk were higher than those found in raw cow's milk, which can be justified mainly by the level of hygiene in the process of obtaining and milking milk.

**Table 1** Distribution of total bacterial count in raw goat's milk samples (bulk tank and individual) in Slovakia (n = 1,054)

| Parameter | Value |
|---|---|
| Geometric mean | $5.4x10^4$ CFU/mL |
| Arithmetic mean | $2.7x10^5$ CFU/mL |
| Percentage of samples < $1.0x10^3$ | 10.9 % |
| Percentage of samples ($1.0x10^3$ – $5.0x10^5$) CFU/mL | 68.6 % |
| Percentage of samples ($5.1x10^5$ – $1.5x10^6$) CFU/mL | 10.4 % |
| Percentage of samples ($1.6x10^6$ – $2.9x10^6$) CFU/mL | 3.2 % |
| Percentage of samples > $3.0x10^6$ CFU/mL | 6.9 % |

Somatic cell count was also measured in raw goat's milk samples. Although this property is not a mandatory health safety indicator for non-cow milks, it may affect the reliability of the recalculation of microbiological quality results, as discussed later. In the table 2 shows a summary of the results of measurements of the number of somatic cells in raw goat's milk. 79.1% of all milk samples were measured in the measurement range of 20,000 to 2,000,000 /mL (it is determined by the limits of the used Fossomatic 7 analyser calibration), 20.1% of the analysed milk samples were outside the measurement range. 48% of samples were measured up to 1,000,000 /mL. The geometric mean of somatic cell count reached values of 1,049,000/ml, or arithmetic mean 1,661,000 /ml. It is clear from the given data that the measured somatic cell count in raw goat´s milk was significantly higher than those found in undisturbed raw cow's milk. In non-cow milks, however, it is common and similar results were reported by **Podhorecká *et al.* (2021).**

**Table 2** Distribution of somatic cell count in raw goat's milk samples (bulk tank and individual) in Slovakia (n = 1 182)

| Parameter | Value |
|---|---|
| Geometric mean | 1,049,000 /mL |
| Arithmetic mean | 1,661,000 /mL |
| Percentage of samples < 499,999 /mL | 20.8 % |
| Percentage of samples (500,000 – 999,999) /mL | 27.2 % |
| Percentage of samples (1,000,000 – 1,499,999) /mL | 12.7 % |
| Percentage of samples (1,500,000 – 1,999,999) /mL | 18.4 % |
| Percentage of samples >2,000,000 /mL | 20.9 % |

**Conversion of the results from laser flow cytometry into CFU/mL scale**

The BactoScan FC laser flow cytometer equipped with the FOSS Integrator software allows you to measure raw milk samples in two measurement modes – normal and enhanced. The enhanced mode consists in the fact that the flow of the measured sample through the measuring cell is slower, which makes it possible to partially eliminate potentially interfering components of the samples. This mode is recommended to be used especially for samples of raw sheep's and goat's milk. As has been proven, raw goat's milk contains relatively high numbers of somatic cells **(Tomáška et al., 2023b)** and these can affect the measurement itself **(Tomáška et al., 2023a)**. Therefore, to establish a conversion relationship, raw goat's milk samples were measured on the BactoScan FC device in this mode.
BactoScan FC measures raw milk samples primarily in Individual Bacterial Cells (IBC) units. It is a proprietary expression of the results of the FOSS company and means the number of pulses of stained and laser-irradiated cells counted in the detector, above the noise limit. The values of the microbiological quality results in these units are therefore different compared to the results of the plate culture method in CFU units. The conversion equation of the total bacterial count of raw goat's milk measured by the laser flow cytometry method (IBC/µL) to the CFU/mL scale is shown in in the figure 1.

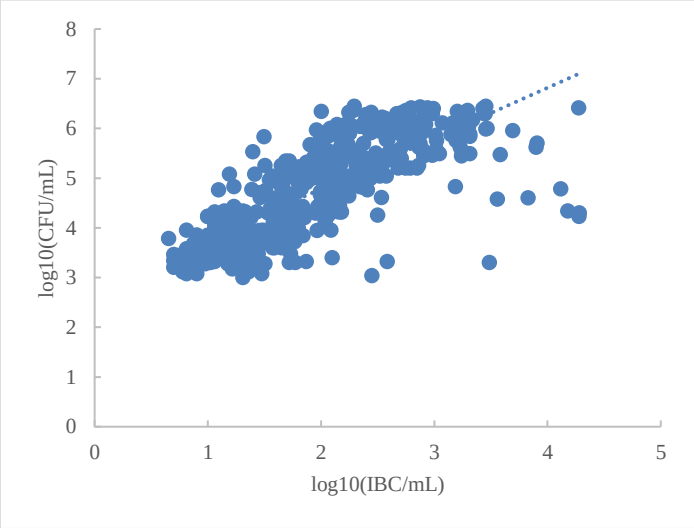


**Figure 1** Conversion of total bacterial count results in raw goat's milk measured by the flow cytometry method (x) into the CFU/mL scale (y) (n = 510)

The conversion, calculated from a set of results with the number of n = 510 in the form of a linear equation, had the form:
$\log_{10}(CFU/mL) = 1.0174 \times \log_{10}(IBC/\mu l) + 2.7483$,
while the correlation coefficient reached the value R=0.76 and the standard deviation regression reached the value of $s_{y,x}$=0.63 $\log_{10}$(CFU/mL).
In comparison with the conversion equation created for raw cow's and sheep's milk in Slovakia **(Tomáška and Suhren, 2004; Tomáška *et al.*, 2006)**, this one was different. It confirms that the type of milk affects the values of coefficients a and b in the linear regression equation. It is also clear that, compared to raw cow's milk, the values of the correlation coefficient are lower for non-cow milk and, conversely, the values of the standard deviation of the regression are larger. As can be seen from the value of correlation coefficient, as well as from Figure 1, the correlation between the two quantities was not ideal and was worse than similar calculations used for raw cow's and sheep's milk. For this reason, the value of standard deviation of regression was relatively high, which means that when measuring total bacterial count in raw goat's milk by the laser flow cytometry method, a larger combined uncertainty of measurement must be taken into account, which is calculated as 1.96 x $s_{y,x}$.
**Ramsahoi *et al*. (2011)** in a similar study conducted with raw goat's milk in Canada understandably arrived at a conversion relationship that differed from that published here. But they also confirmed that the conversion relationships for raw

cow's milk and raw goat's milk are different. In Canada, the legislative limit for quality milk is 50,000 CFU/mL, which in IBC units was 121,000 IBC/mL for raw cow's milk, and up to 321,000 IBC/mL for raw goat's milk. The authors explain this by saying that, unlike raw cow's milk, raw goat's milk is collected in bulk tanks for one and a half to two times longer. As for the calculation model used, the authors concluded that the recalculation of the results did not follow a linear course throughout. For this reason, it is possible that the conversion relationship created for the conditions of Slovakia will be calculated in the future during its further verification, after supplementing the relevant data, with just such a model. Another possible way to improve the correlation of variables in the conversion equation is to cut off results above about 3.5 $\log_{10}$ (IBC/µL). It is clear from the Figure 1 that with such IBC counts, the measured values in CFU were below the linear regression line. This may have been due to the presence of dead or damaged cells in these samples that were measured by the BactoScan FC but did not grow in the plate-culture method. After the reduction of these samples, of course, the conversion relation changed, and the correlation coefficient rose to the value R=0.83.

**Verification of the conversion equation**

To verify the created conversion, individual and pool samples (215 samples) of raw goat's milk measured obtained from 7 producers and processors from Slovakia in 2023 were used. The results measured by laser flow cytometry were converted to the CFU/mL scale using the created conversion. Subsequently, the recalculated results and directly measured results by plate-cultivation method were compared and statistically evaluated.

Directly measured total bacterial count values ranged from $<1x10^3$ CFU/mL to $>3x10^6$ CFU/mL. After excluding the results that can be considered uncountable from the point of view of the methodology according to **STN EN ISO 4833-1**, the arithmetic mean of the given set of results reached the value of $176x10^3$ CFU/mL and the geometric mean of the results reached the value of $52x10^3$ CFU/mL. Somatic cell count in the given set of samples was in the range of 2,600 – 13,700,000 /mL, the arithmetic mean reached the value 1,552,000 /mL and the geometric mean the value 919,000 /mL. The set of results used for verification can therefore be considered representative.

As for the actual differences found between directly measured and recalculated total bacterial count values in CFU/mL units, they were as follows: the average difference between directly measured values and recalculated values was -0.09 $\log_{10}$(CFU/mL) and the standard deviation of the differences was 0.56 $\log_{10}$(CFU/mL). Both values are therefore smaller than the calculated value of standard deviation of regression. Of course, since the principle of the laser flow cytometry method is completely different from that of the cultivation method, there were also bigger differences observed – the absolute value of the maximum difference was 2.64 $\log_{10}$(CFU/mL). Larger differences were measured especially at higher numbers of IBCs, as can be seen in the figure 2.

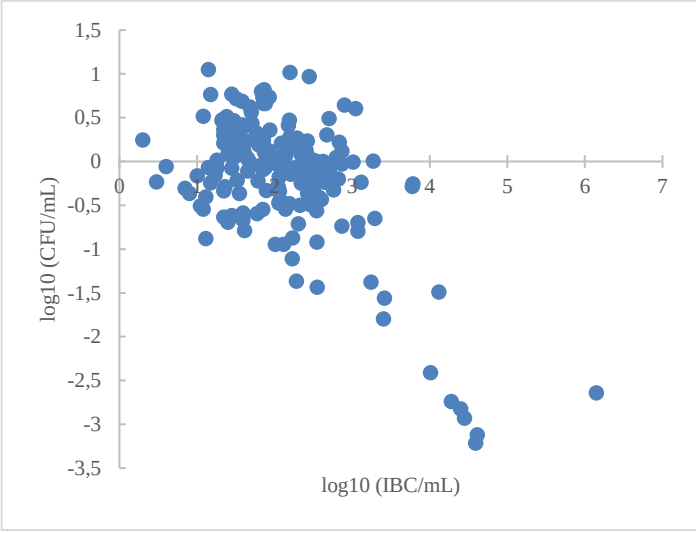


**Figure 2** Magnitude of differences between directly measured total bacterial count and recalculated results from the laser flow cytometry method (y) by individual bacterial counts (x) in raw goat milk samples

During the measurement of results in 2023, it was confirmed that high somatic cell count can negatively affect the measurement of total bacterial count by the laser flow cytometry method. However, when comparing the directly measured total bacterial count results and the converted results, the magnitude of the differences was not found to be directly related to the somatic cell counts in the samples, as shown in the figure 3. Thus, the enhanced mode on the BactoScan FC device has its advantages in the case of raw goat milk samples.

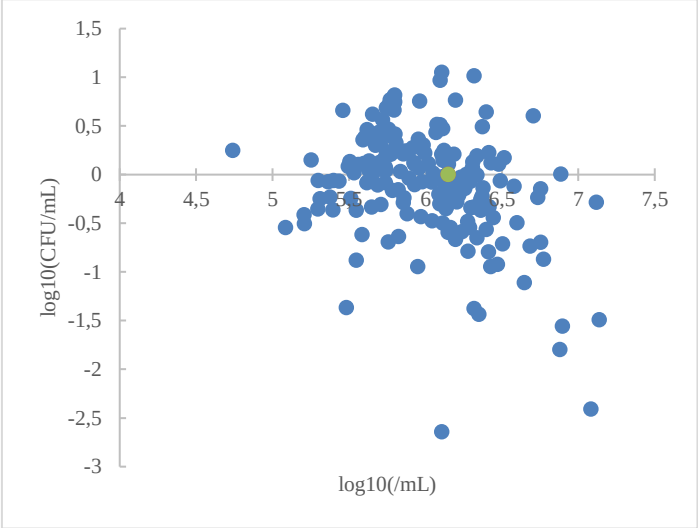


**Figure 3** Magnitude of differences between directly measured total bacterial count and recalculated results from the laser flow cytometry method (y) by somatic cell count (x) in raw goat milk samples

With laser flow cytometry, the verification of the created calculation is a permanent process**. ISO 21187** recommends, after measuring a new set of results, to add them to the original set and to update the conversion equation after a certain time, thus ensuring its up-to-datedness and reliability.

**CONCLUSION**

Evaluation of the microbiological quality of raw goat's milk using laser flow cytometry is feasible in Slovakia. The method offers its indisputable advantages, primarily the speed of obtaining results and the possibility of automation. The created conversion equation for converting directly measured results to the CFU/mL scale is representative for Slovakia. However, compared to similar conversions for raw cow's and sheep's milk, it is necessary to consider greater measurement uncertainty, and therefore it can be expected in practice that critical samples (with values close to the hygienic acceptability limit, counter-samples, etc.) will be verified by the reference plate method. Although the use of the enhanced mode on the BactoScan FC flow cytometer greatly eliminates the risk of interference from higher somatic cell count on the measurement itself, it is also recommended to use the reference plate method for the determination of total bacterial count for samples with very high somatic cell count.

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