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A comprehensive assessment of the quality of raw cow's milk based on somatic cell count and the presence of mesophilic aerobic and facultative anaerobic microorganisms as indicators of the safety and processing properties of raw milk

Togachynska L. (ORCID: 0009-0005-5032-5940), Kuriata N. (ORCID: 0000-0002-6958-1064), Musiiets I. (ORCID: 0000-0002-2456-560X), Pishchansky O. (ORCID: 0009-0002-0111-4977), Halka I. (ORCID: 0000-0001-8701-3506), Balanchuk L. (ORCID: 0000-0003-0989-5886), Kulykova V. (ORCID:0009-0008-8827-030X)

State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine, e-mail: tog.liya888@gmail.com

Abstract. *This study conducted a comparative evaluation of methods for determining the somatic cell count and the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw cow's milk. The aim of the study was to establish the analytical agreement, accuracy, and reproducibility of results obtained by different methods, as well as to justify the feasibility of using the TEMPO® automated system as an alternative method for microbiological control.*

The study material consisted of 39 samples of raw cow's milk collected in 2025 from clinically healthy Black-and-White and Red dairy cows aged 4–8 years on private farms in various regions of Ukraine. Somatic cell counts were determined using two microscopic methods in accordance with the requirements of ISO 13366-1:2008: by counting cells in smears across fifty fields of view and in ten parallel strips. The number of total aerobic bacteria was determined using two independent methods: an automated fluorometric method using the TEMPO® system and a reference method of deep plating on Plate Count Agar medium in accordance with ISO 4833-1:2013.

Statistical analysis of the results was performed using Student's paired t-test and Pearson's correlation analysis. It was found that the mean values of somatic cell counts obtained by different methods were 301,795 and 294,641 cells/cm³, respectively, and for MAFAnM—43,041 and 43,513 CFU/cm³. The calculated t-test values ($t = 1.80$ for somatic cells and $t = -1.32$ for MAFAnM) did not exceed critical values ($p > 0.05$), indicating the absence of statistically significant differences between the methods. The Pearson correlation coefficient was $r = 1.0$ for somatic cells and $r = 0.9996$ for MAFAnM ($p < 0.05$), indicating a strong direct linear relationship and high consistency of results.

The research demonstrated that TEMPO® automated system provides results for the detection of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw milk that are statistically equivalent to the reference method, with high accuracy and reproducibility and minimal human influence. The use of this system is recommended for implementation in production laboratories as a rapid and reliable tool for monitoring the microbiological parameters of raw milk.

Keywords: somatic cells, mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), cow's milk, species, automated TEMPO® method, correlation analysis, Student's t-test.

Cow's milk is one of the most valuable and nutritionally balanced foods in the human diet, as it contains a wide range of nutrients essential for the body's normal functioning. It is characterized by high nutritional and biological value due to its optimal combination of proteins, fats, carbohydrates, minerals, and vitamins. Milk proteins consist of casein and whey proteins, which are complete proteins containing all the essential amino acids necessary for the synthesis of body tissues, with a digestibility of up to 95–98%. Milk fat is a source of energy and a carrier of fat-soluble vitamins, while lactose meets the body's energy needs and promotes better calcium absorption (Vranješ, 2015; Mykhailiutenko, 2025).

Milk is particularly important as a source of minerals, specifically calcium and phosphorus, which are present in an optimal ratio that supports the effective formation and maintenance of bone tissue and teeth. The presence of vitamin D further enhances calcium absorption, which is important for children during periods of rapid growth and for adults to help prevent osteoporosis. In addition, milk contains B vitamins, which have a positive effect on nervous system function, as well as potassium, which helps regulate water-salt balance and blood pressure (Li, 2025).

The food industry makes extensive use of cow's and goat's milk, as well as dairy products (hard and processed cheeses, butter, fermented milk products, etc.). Milk and dairy products are an important part of the human diet (Sadvari, 2024).

The nutritional value of cow's milk is also due to the presence of biologically active components, such as enzymes, immunoglobulins, lysozyme, and lactoferrin, which play an important role in building the body's immune defenses and maintaining normal intestinal microflora. As a result, milk helps increase the body's resistance to infectious diseases (Miftari, 2026).

Cow's milk is an accessible source of energy and nutrients, making it particularly important in the diets of children, adolescents, individuals with high physical activity levels, and the elderly. At the same time, individual physiological characteristics should be taken into account, as some people may have lactose intolerance or allergic reactions to milk proteins. Thus, when consumed in moderation, cow's milk is an important component of a balanced diet and contributes to maintaining good health (Zhao, 2025).

The dairy industry in Ukraine is of great economic importance, as it provides the population with essential food products of high nutritional value. The growing demand for dairy products in Ukraine is confirmed by statistical data, which indicates stable consumption of milk and dairy products. This demonstrates the continued importance of dairy products in the diet of citizens, which in turn stimulates the development of the dairy industry, as well as milk production and processing. The high quality of milk and dairy products directly impacts the industry's profitability and its competitiveness in domestic and international markets (Yang, 2025; Curci, 2025).

The primary goal of dairy farms is to provide environmental conditions that meet the needs of the breed being raised in order to support profitable and sustainable production. Given that cattle productivity is the result of a complex interaction between genotype and environmental conditions, it is crucial to provide appropriate environmental conditions to enhance productivity. Sustainable and profitable production depends on the number of calves born per year and optimal milk yield from each cow. Around the world, breeding research on dairy cows has focused primarily on increasing milk yield for many years. However, most researchers note that increased milk yield has a negative impact on the fertility of dairy cows. Additionally, the elevated metabolic rate in high-producing cows tends to negatively affect their metabolic status. A fast metabolism can lead to an imbalance in energy and nutrients (Ermetin, 2025; Nuzzi, 2026).

Because milk production is the primary source of income for dairy farms, it is important to consider factors that can directly affect milk production, such as mastitis. Mastitis is the most common disease of the mammary gland in cattle, goats, sheep, and other animals (Nagy, 2025).

Mastitis is an inflammatory disease of the mammary gland in animals, usually caused by a bacterial infection, although other causes (trauma, stress, poor hygiene) may also be involved. It results in significant economic losses in the dairy industry due to its direct impact on milk production. Mastitis is a major problem in terms of reduced productivity and the quality and safety of milk. Somatic cells serve as a criterion for determining the quality and safety of raw milk and the health status of the mammary gland in animals (Kotelevich, 2023; Kukeyeva, 2023).

The somatic cell count is widely used as an indicator of udder inflammation, since both subclinical and clinical mastitis result in high levels of these cells.

Somatic cells in raw cow's milk are an important biological and diagnostic indicator that characterizes the physiological condition of the mammary gland, the sanitary quality of milk, and its technological properties. The term "somatic cells" refers to the totality of cells present in milk, which include, on the one hand, mammary gland epithelial cells that are shed during milk secretion, and on the other hand, cells of the immune system (leukocytes) (Fonseca, 2025; Fonseca, 2025).

Leukocytes are vital to the immune system of animals, playing a key role in protecting the mammary gland from environmental pathogens, including bacteria, viruses, and fungi. The composition and relative proportions of these leukocytes vary among different animal species, reflecting the unique immune response mechanisms of different breeds. Although all animals have the same basic types of white blood cells (neutrophils, monocytes, eosinophils, and basophils). In cattle, lymphocytes predominate—they account for approximately 50–70%, while neutrophils are less common (20–40%). This type of blood is called lymphocytic (Desidera, 2025).

In physiologically healthy cows, the somatic cell count in milk is relatively low and falls within the limits established by regulatory documents, indicating a normal udder condition and the absence of inflammatory processes. The majority of these cells are macrophages and epithelial cells, which perform protective and regulatory functions. Macrophages participate in the phagocytosis of microorganisms and cellular debris, providing local immunity, while epithelial cells reflect the physiological renewal of mammary gland tissues (Besteiro, 2025).

When inflammatory processes develop, particularly mastitis, there is a sharp increase in the number of somatic cells in milk, which is caused by the migration of leukocytes to the site of infection. In such cases, neutrophils become the dominant cell type, playing a key role in neutralizing pathogenic microorganisms. The increase in somatic cell count is a protective response of the body; however, it is accompanied by changes in the physical, chemical, and technological properties of milk (Pan, 2025).

Elevated somatic cell counts negatively affect milk quality; specifically, they lead to a decrease in casein content, an imbalance in protein fractions, and increased activity of proteolytic and lipolytic enzymes, which causes the hydrolysis of proteins and fats. This, in turn, impairs the taste properties of milk, reduces its heat stability and processing suitability, particularly in the production of cheese and fermented milk products. Furthermore, a high somatic cell count may indicate the presence of pathogenic microorganisms and a decline in the sanitary quality of the raw material (Mikulec, 2024; Smistad, 2025).

From a diagnostic standpoint, determining somatic cell count is one of the key criteria for monitoring udder health and detecting subclinical mastitis at an early stage. This parameter is widely used in veterinary practice and the dairy industry as an indicator of raw milk quality and compliance with regulatory requirements.

Thus, somatic cells in raw cow's milk play a dual role: on the one hand, they are a key component of the mammary gland's local immune defense, and on the other, they serve as a sensitive indicator of the gland's physiological condition and milk quality. Controlling their numbers is crucial for ensuring the safety, nutritional value, and technological suitability of raw milk (Mikulec, 2024).

The analysis of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw cow's milk plays a different role. It is one of the main microbiological indicators characterizing its sanitary quality, safety, and technological suitability. It characterizes the level of milk contamination and provides an assessment of milking hygiene and equipment cleanliness. Unlike somatic cells, the MAFAnM count is not a direct indicator of udder health. A high MAFAnM count may result from external contamination, even in animals with healthy udders. At the same time, the number of microorganisms may increase in the presence of mastitis, but this is not always the case and does not always correlate directly (Ryzhkova, 2023).

MAFAnM consists of various groups of microorganisms capable of growing under aerobic or facultatively anaerobic conditions at a temperature of approximately 30 °C. It comprises mainly saprophytic bacteria, as well as opportunistic pathogens that may enter milk from the environment.

The main sources of milk contamination, as measured by the MAFAnM index, include the animal's udder surface, milking equipment, tools, water, air, and the hands of the staff. In addition, microorganisms can enter the milk if sanitary and hygiene requirements are not met during milking, transportation, and storage. An important factor affecting the MAFAnM level is storage temperature: if milk is not cooled promptly, conditions favorable for the rapid multiplication of microorganisms are created (Ryzhkova, 2023).

This indicator provides a comprehensive assessment of the overall microbial contamination of milk and is widely used in laboratory practice for quality control. A low level of total bacterial count indicates proper sanitary conditions in production, compliance with hygiene requirements during milking, and effective milk cooling. Such milk is characterized by high storage stability, preservation of organoleptic properties, and suitability for further processing. Conversely, a high MAFAnM content is a sign of microbial contamination and a disruption in the production process, which can lead to the rapid growth of microflora (Yuan, 2022).

An increase in the number of MAFAnM is accompanied by an intensification of enzymatic processes, particularly proteolysis and lipolysis, which cause the breakdown of milk proteins and fats. This leads to the formation of decomposition products that impair the taste, odor, and

consistency of milk, reduce its nutritional value, and shorten its shelf life. In addition, high microbial contamination of milk can complicate technological processes, particularly pasteurization and the production of dairy products, since some microorganisms or their enzymes may remain active even after heat treatment (Ryzhkova, 2023).

From a safety perspective, the MAFAnM value is important as an indicator of the overall sanitary condition of milk, although it does not provide direct information about the presence of specific pathogenic microorganisms. However, high MAFAnM values increase the likelihood of the presence of undesirable or potentially hazardous microflora, which may pose a risk to consumer health. Regular monitoring of this indicator allows for the timely detection of deviations in the production process, the prediction of milk shelf life, and the assurance of its compliance with established regulatory requirements (Micules, 2025).

Thus, the analysis of somatic cells allows for an assessment of the udder's health, while the count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) reflects the sanitary and hygienic standards of milk production. Both indicators are important and are used in conjunction to provide a comprehensive assessment of raw milk quality.

Materials and methods. The experimental studies were conducted at the Laboratory for Microbiological Analysis of Food and Feed within the Research Bacteriology Department of the State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (DNDILDVSE), which is accredited by the National Accreditation Agency of Ukraine for competence in accordance with the requirements of DSTU ISO/IEC 17025-21, Kyiv.

The study utilized samples of raw cow's milk collected throughout 2025 from private farms located in various regions of Ukraine. The milk was obtained from clinically healthy animals. At the time of sampling, the cows were not pregnant and were not in the lactation period, which helped minimize the influence of physiological factors on milk composition. Cow's milk was obtained from Black-and-White and Red dairy breeds aged 4 to 8 years. The animals were kept on private farms and were in a physiologically normal condition. The fat content in cow's milk averaged 3.2 g per 100 g of product (3.2%). The collected raw milk samples were transported to the laboratory in special insulated containers with cooling packs, which ensured that the temperature was maintained $+4\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

During the research, 39 samples of raw milk were analyzed. The study was conducted based on two main parameters:

- determination of somatic cell count;
- determination of the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM).

The somatic cell count was determined using two microscopic methods (to compare results) by counting cells in stained smears in accordance with the requirements of the international standard ISO 13366-1:2008 (ISO 13366, 2008).

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw milk samples was determined using two independent methods for the purpose of comparing the results. The first method involved the use of the TEMPO® automated system. The second method for determining MAFAnM was performed in accordance with the requirements of ISO 4833:2013 (ISO 4833, 2013).

Prior to microscopic examination, the milk was first incubated in a water bath at $+40\text{ }^{\circ}\text{C}$ for 20 minutes to determine somatic cell count; it was then cooled to room temperature and thoroughly mixed to ensure the homogeneity of the sample. For each test sample, at least two microscopic slides were prepared, from which the slide of the highest quality was selected for counting.

To prepare a smear, 0.01 cm^3 of pre-mixed milk was applied to a clean microscope slide using a microsyringe. The drop was placed within a pre-marked area measuring $1\text{ cm}^3 \pm 5\%$ (from 95 mm^2 to 105 mm^2) (Fig. 1).

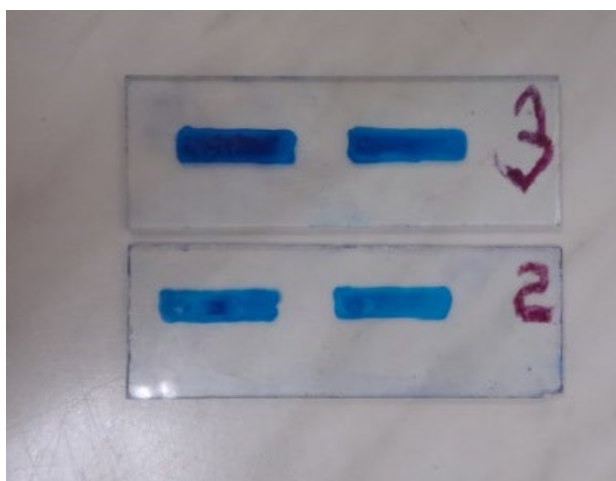


Fig.1 A smear of raw milk stained with a Newman-Lampert dye solution

The material was evenly spread over the surface of the area in a thin layer using a dissecting needle. Afterward, the specimen was left to dry at room temperature until completely dry.

After drying, the slides were immersed in a staining bath containing a modified Newman-Lampert stain and left for 15 minutes. After staining, the slides were removed, dried at room temperature, and then rinsed with distilled water to remove any residual stain.

Somatic cell counts (leukocytes, lymphocytes, monocytes, and epithelial cells) were performed using a microscope at the most appropriate magnification (ranging from 500 \times to 1000 \times), (Fig. 2).

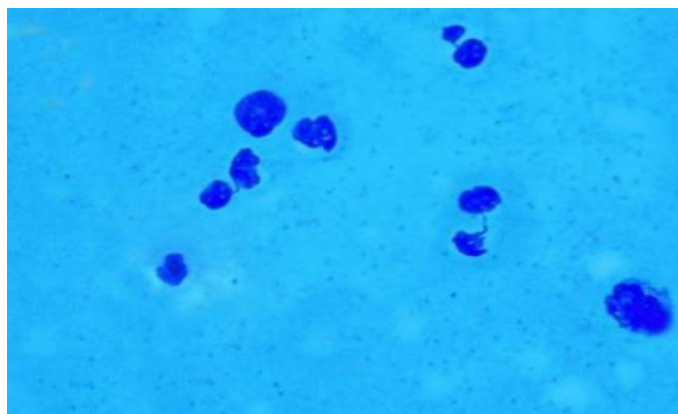


Fig. 2. Somatic cells in the smear (magnification $\times 1000$)

The obtained values were then used to calculate their concentration in the tested milk sample.

To determine the number of mesophilic aerobic and facultative anaerobic microorganisms in raw milk using the TEMPO® system, the test samples were prepared in stages. From each batch of test material, 10 cm³ of raw milk was sampled and aseptically transferred into a sterile filter bag (Bag Filter). We added 90 cm³ of sterile primary solvent—peptone water—to the bag, resulting in an initial dilution of 1:10. The resulting suspension was thoroughly homogenized using a homogenizer (Bag Mixer) until a homogeneous mixture suitable for further processing was formed.

Using the dispenser on the TEMPO® Aerobic Count unit, 9 ml of secondary solvent (saline solution) and 1 ml of the sample from the primary dilution were added to the vial containing the dry culture medium to fully dissolve the dry culture medium and prepare the mixture for further analysis (Fig. 3).



Fig. 3. Dry growth medium TEMPO® AC

The mixture was homogenized for 3 seconds.

Further preparation for analysis was performed using the TEMPO® FILLER sample preparation station (Fig. 4).



Fig. 4. TEMPO® Filler Sample Preparation Station (card filling and sealing)

After logging into the instrument's software, the corresponding working module was opened to enter information about the test samples.

The identification data for each sample were read using a barcode scanner. Information such as the sample number, test date, type of matrix being tested, and the selected analytical method was automatically entered into the system.

Special TEMPO® AC cards, designed for determining MAFAnM, were attached to each vial containing the prepared inoculated culture medium. Before starting the procedure, the barcodes on the vial and the card were verified by scanning them. After that, the vials and cards were placed in a rack. One rack is designed to hold up to six vials and six cards at a time.

The prepared rack was placed into the TEMPO® FILLER device, where the card wells were automatically filled with the inoculated culture medium. During this process, the contents of the vial were completely drawn into the card's micro-well system. After the card filling process was complete, the TEMPO® FILLER automatically cut off the transport tubes and hermetically sealed the cards (Fig. 5).

After the approximately three-minute cycle was complete, the rack with the filled cards was removed from the sample preparation station. An empty vial indicated that the inoculated medium had been completely and correctly transferred to the card.

The prepared plates were placed in a special incubation rack, oriented so that the labels faced the rack handle. The plates were then transferred to a thermostat, where the microorganisms were cultured at a temperature of $30\text{ °C} \pm 1\text{ °C}$ for 40–48 hours.

Microbiological examination of the test samples, processing of the obtained data, and interpretation of the results for determining the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) were performed using the second method in accordance with the requirements of the international standard ISO 4833-1:2013.

Buffered peptone water was used to prepare the initial suspension in a 1:10 ratio. When using the standard method, a series of consecutive decimal dilutions of the test material (10^2 , 10^3 , etc.)

was prepared. From the corresponding dilutions, 1 cm³ of suspension was collected using a sterile pipette and transferred to sterile Petri dishes.



Fig. 5. Cards with cut-off straws

To each Petri dish containing the material, approximately 15 cm³ of Plate Count Agar medium was added, which had been pre-cooled in a water bath to a temperature of (47 ± 2) °C. After that, the contents of the dishes were gently mixed with light circular motions to ensure uniform distribution of the sample in the medium.

After the agar medium had completely solidified, the plates were incubated in a thermostat at a temperature of (30 ± 1) °C for (72 ± 3) hours. Upon completion of incubation, all microbial colonies on the plates suitable for counting were counted, i.e., those containing between 15 and 300 colonies on at least one of the plates of the corresponding dilution.

Results and Discussion. A study was conducted on 39 samples of raw milk to determine somatic cell count and the presence of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM).

Somatic cell counts were determined using two calculation methods:

1) The number of rectangular somatic cells in the smear was counted and calculated in fifty consecutive, evenly spaced fields of view using the following formula:

$$C = \frac{W_S \times L_S \times N_t}{\pi \times \left(\frac{D_f}{2}\right)^2 \times N_f \times V_m} \times \frac{1}{d},$$

where:

C - total cell concentration per cm³;

W_S - horizontal diameter of the smear, mm;

L_S - vertical diameter of the smear, mm;

N_t - total number of cells counted;

D_f - diameter of the microscope's field of view, mm;

N_f - number of all fields of view in which cells were counted;

V_m - volume of the test sample in the smear;

V_m = 0,01 (if the Newman-Lampart colorant was used for coloring);

d - sample dilution factor;

d = 1 (if the sample was not diluted).

2) The number of rectangular somatic cells in the smear was counted and calculated in consecutive fields across ten parallel vertical strips using the following formula:

$$C = \frac{L_S \times N_t}{D_f \times N_b \times V_m} \times \frac{1}{d},$$

with:

N_b - the total number of counted lanes.

Calculations of the somatic cell count in the test samples showed that both calculation methods are reliable and practical for use (Fig. 6), (Table 1).

The study and counting of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) were conducted using the automated TEMPO® method and the reference method of deep plating on Petri dishes containing Plate Count Agar medium.

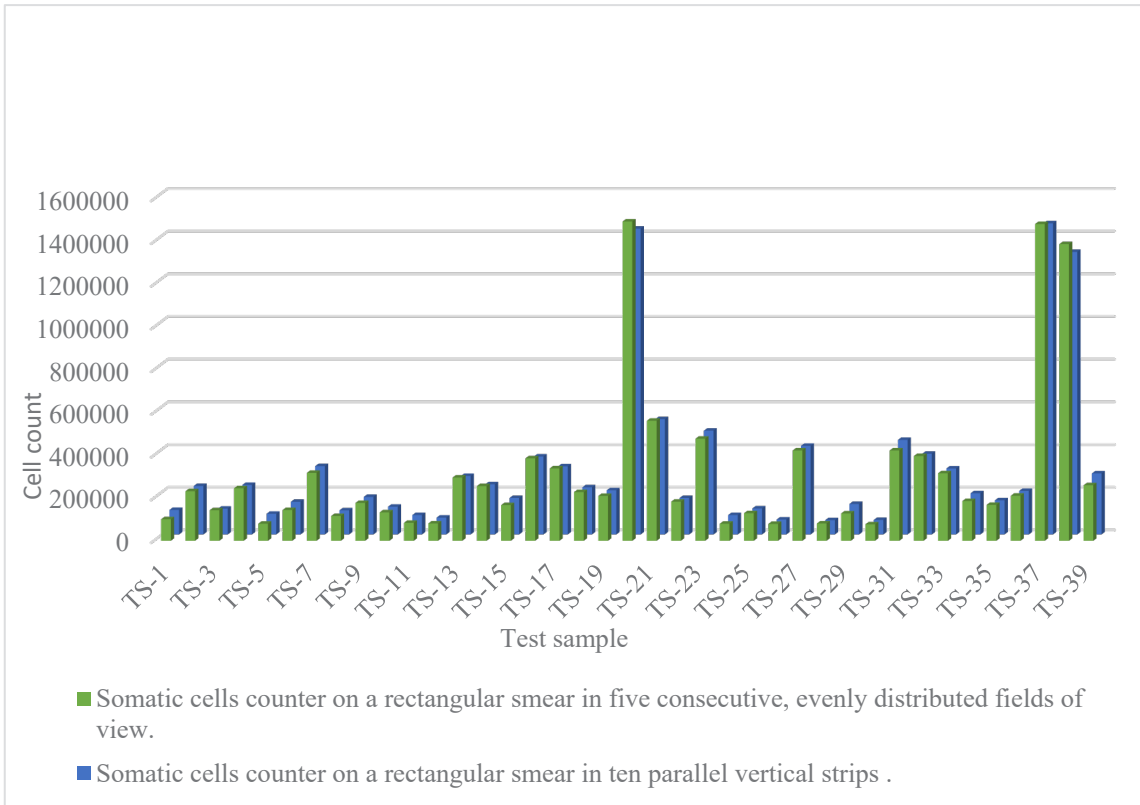


Fig. 6. Results of somatic cell count in raw milk using two calculation approaches

After incubation, the TEMPO® AC cards used in the study at the MAFAnM via the automated TEMPO® method were scanned to interpret the results. A rack containing the cards was placed in the TEMPO® READER (card reading station). The device automatically read the barcode on each card. Next, the fluorescence intensity in individual wells of the card was measured. Based on the obtained fluorescence signals, the software processed the data, calculated the quantitative content of mesophilic aerobic and facultative anaerobic microorganisms in the samples, and displayed the results electronically for further interpretation.

In individual wells of the card, the system automatically identified positive and negative wells and performed mathematical calculations to determine the bacterial concentration in the samples. The results were then recorded.

The calculated test results were displayed on the computer screen, converted to the original product in CFU/cm³, taking into account the dilutions applied, which ensured an accurate representation of the quantitative content of microorganisms in the sample (Fig. 2), (Table 2).

Following the inoculation of samples onto Petri dishes containing Plate Count Agar, characteristic colonies were observed to grow. The colonies were white to cream in color; yellowish-gray colonies were also noted. The surface of the colonies was smooth, and their consistency was soft and slimy (Fig. 7).



Fig. 7. Growth of MAFAnM colonies on the medium Plate Count Agar

Table 1

Analysis of the results of a study on the somatic cell count in raw milk ($M \pm m$, $n = 5$)

Test samples (TS)	Results of the count of rectangular somatic cells in a smear across fifty consecutive, evenly spaced fields of view, cells/cm ³	Results of the count of rectangular somatic cells in a smear across consecutive fields on ten parallel vertical strips, cells/cm ³
TS № 1	101 000 ± 31	115 000 ± 35
TS № 2	232000 ± 69,0	227 000 ± 68
TS № 3	143 000 ± 43	121 000 ± 36
TS № 4	246 000 ± 74	232 000 ± 70
TS № 5	80 000 ± 24,0	97 000 ± 29
TS № 6	144 000 ± 43	153 000 ± 46
TS № 7	318 000 ± 95	320 000 ± 96
TS № 8	116 000 ± 35	113 000 ± 34
TS № 9	177 000 ± 53	176 000 ± 53
TS № 10	133 000 ± 40	130 000 ± 39
TS № 11	84 000 ± 42	91 000 ± 27
TS № 12	81 000 ± 24	79 000 ± 24
TS № 13	296 000 ± 89	274 000 ± 82
TS № 14	256 000 ± 77	235 000 ± 71
TS № 15	167 000 ± 50	171 000 ± 51
TS № 16	386 000 ± 116	365 000 ± 110
TS № 17	339 000 ± 102	319 000 ± 96
TS № 18	228 000 ± 68	221 000 ± 66
TS № 19	211 000 ± 63	207 000 ± 62
TS № 20	1495 000 ± 299	1432 000 ± 430
TS № 21	562 000 ± 169	540 000 ± 162
TS № 22	183 000 ± 55	171 000 ± 51
TS № 23	478 000 ± 143	486 000 ± 146
TS № 24	80 000 ± 24	91 000 ± 27
TS № 25	129 000 ± 39	122 000 ± 37
TS № 26	79 000 ± 24	70 000 ± 21
TS № 27	423 000 ± 127	415 000 ± 125
TS № 28	81 000 ± 24	67 000 ± 20
TS № 29	128 000 ± 39	143 000 ± 43
TS №30	77 000 ± 23	68 000 ± 20
TS №31	423 000 ± 127	443 000 ± 133
TS №32	397 000 ± 119	378 000 ± 113
TS № 33	316 000 ± 95	309 000 ± 93
TS № 34	186 000 ± 56	193 000 ± 58
TS № 35	168 000 ± 50	160 000 ± 48
TS № 36	211 000 ± 63	204 000 ± 61
TS № 37	1483 000 ± 445	1457 000 ± 437
TS № 38	1389 000 ± 417	1323 000 ± 397
TS № 39	260 000 ± 78	286 000 ± 86

 $p < 0,05$

Section 2

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) that grew on Petri dishes in the test samples was calculated using the appropriate formula.

$$N = \frac{\sum c}{(n_1 + 0,1 \cdot n_2) \cdot d},$$

with:

N - number of microorganisms (CFU/cm³);

$\sum c$ - the total number of colonies counted in all Petri dishes;

n_1 - the number of cups in which colonies grew in the first culture;

n_2 - the number of cups in which colonies grew in the second culture;

d - dilution ratio.

The results obtained regarding the number of MAFAnM were expressed in colony-forming units (CFU/cm³) (Fig. 8), (Table 2).

A comparison was conducted of selected values from the study of somatic cell count and the number of MAFAnM present in raw milk.

The somatic cell count results were compared between the two counting methods in rectangular smears, specifically across fifty consecutive, evenly spaced fields of view and across ten parallel vertical strips.

For this purpose, Student's t-test for paired samples was used, which allows comparing the means of two dependent samples and determining whether they differ statistically significantly.

The study included 39 paired samples, for each of which the analyses were performed using both methods simultaneously.

Calculations of Student's t-test (t) to assess differences between the results of determining somatic cell count and the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in raw milk were performed using a paired t-test, since the results obtained for the same samples using different methods were being compared. The calculation was performed using the formula:

$$\bar{d} = \frac{1}{n} \sum_{i=1}^n (x_i - y_i),$$

$$\bar{d} \approx 7230,77 \text{ somatic cells/cm}^3$$

$$\bar{d} \approx -217,95 \text{ CFU/cm}^3 \text{ (MAFAnM)},$$

where x_i - is the number of somatic cells counted in rectangular smears across fifty consecutive, evenly spaced fields of view (cells/cm³);

y_i is the number of somatic cells counted in rectangular smears across ten parallel vertical strips (cells/cm³);

d - the standard deviation of the values between the two methods;

n - the number of test samples

Standard deviation:

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}},$$

$$S_1 \approx 44725 \text{ somatic cells /cm}^3;$$

$$S_2 \approx 42462 \text{ somatic cells /cm}^3;$$

$$S_1 \approx 36360 \text{ CFU/cm}^3 \text{ (MAFAnM)}$$

$$S_2 \approx 36630 \text{ CFU/cm}^3 \text{ (MAFAnM)}$$

where,

S - standard deviation, which characterizes the degree of variability (dispersion) of the results of somatic cell count and MAFAnM measurements relative to their arithmetic mean;

x - the arithmetic mean of the indicator under study, calculated based on the aggregate of the results obtained:

- for somatic cells—based on the results of two counts;
- for MAFAnM - based on the results obtained using different research methods (the automated TEMPO® method and the reference method of inoculation on PCA agar);

x_i - a separate (individual) value of the indicator under study:

for somatic cells—the value obtained from two different counts;

Table 2

**Analysis of the results of the study on the concentration of MAFAnM
in raw milk ($M \pm m$, $n = 5$)**

Test samples	Results of the determination of the concentration of MAFAnM in raw milk using the TEMPO® system, CFU/cm³	Results of the determination of the number of total aerobic bacteria in raw milk using the standard method on Plate Count Agar, CFU/cm³
TS № 1	$(1,2 \pm 0,006) \times 10^4$ CFU/cm ³	$(1,30 \pm 0,017) \times 10^4$ CFU/cm ³
TS № 2	$(3,2 \pm 0,016) \times 10^4$ CFU/cm ³	$(3,3 \pm 0,013) \times 10^4$ CFU/cm ³
TS № 3	$(1,4 \pm 0,007) \times 10^4$ CFU/cm ³	$(1,5 \pm 0,006) \times 10^4$ CFU/cm ³
TS № 4	$(1,0 \pm 0,005) \times 10^3$ CFU/cm ³	$(1,1 \pm 0,003) \times 10^3$ CFU/cm ³
TS № 5	$(1,2 \pm 0,006) \times 10^4$ CFU/cm ³	$(1,1 \pm 0,09) \times 10^4$ CFU/cm ³
TS № 6	$(3,3 \pm 0,016) \times 10^4$ CFU/cm ³	$(3,2 \pm 0,012) \times 10^4$ CFU/cm ³
TS № 7	$(1,9 \pm 0,009) \times 10^4$ CFU/cm ³	$(2,0 \pm 0,008) \times 10^4$ CFU/cm ³
TS № 8	$(3,2 \pm 0,016) \times 10^4$ CFU/cm ³	$(3,3 \pm 0,013) \times 10^4$ CFU/cm ³
TS № 9	$(2,9 \pm 0,014) \times 10^4$ CFU/cm ³	$(3,1 \pm 0,012) \times 10^4$ CFU/cm ³
TS № 10	$(3,7 \pm 0,018) \times 10^4$ CFU/cm ³	$(3,6 \pm 0,0144) \times 10^4$ CFU/cm ³
TS № 11	$(1,1 \pm 0,055) \times 10^5$ CFU/cm ³	$(1,2 \pm 0,044) \times 10^5$ CFU/cm ³
TS № 12	$(2,6 \pm 0,013) \times 10^4$ CFU/cm ³	$(2,7 \pm 0,011) \times 10^4$ CFU/cm ³
TS № 13	$(5,4 \pm 0,027) \times 10^4$ CFU/cm ³	$(5,5 \pm 0,022) \times 10^4$ CFU/cm ³
TS № 14	$(1,5 \pm 0,007) \times 10^4$ CFU/cm ³	$(1,4 \pm 0,005) \times 10^4$ CFU/cm ³
TS № 15	$(3,6 \pm 0,018) \times 10^4$ CFU/cm ³	$(3,7 \pm 0,014) \times 10^4$ CFU/cm ³
TS № 16	$(5,5 \pm 0,027) \times 10^4$ CFU/cm ³	$(5,6 \pm 0,022) \times 10^4$ CFU/cm ³
TS № 17	$(2,0 \pm 0,010) \times 10^4$ CFU/cm ³	$(1,9 \pm 0,007) \times 10^4$ CFU/cm ³
TS № 18	$(1,7 \pm 0,008) \times 10^5$ CFU/cm ³	$(1,8 \pm 0,068) \times 10^5$ CFU/cm ³
TS № 19	$(6,0 \pm 0,030) \times 10^4$ CFU/cm ³	$(5,9 \pm 0,023) \times 10^4$ CFU/cm ³
TS № 20	$(5,4 \pm 0,027) \times 10^4$ CFU/cm ³	$(5,5 \pm 0,022) \times 10^4$ CFU/cm ³
TS № 21	$(1,4 \pm 0,012) \times 10^3$ CFU/cm ³	$(1,5 \pm 0,003) \times 10^3$ CFU/cm ³
TS № 22	$(1,5 \pm 0,007) \times 10^4$ CFU/cm ³	$(1,4 \pm 0,014) \times 10^4$ CFU/cm ³
TS № 23	$(3,7 \pm 0,018) \times 10^4$ CFU/cm ³	$(3,8 \pm 0,012) \times 10^4$ CFU/cm ³
TS № 24	$(9,0 \pm 0,045) \times 10^4$ CFU/cm ³	$(9,1 \pm 0,036) \times 10^4$ CFU/cm ³
TS № 25	$(3,5 \pm 0,017) \times 10^4$ CFU/cm ³	$(3,6 \pm 0,014) \times 10^4$ CFU/cm ³
TS № 26	$(2,1 \pm 0,010) \times 10^4$ CFU/cm ³	$(2,0 \pm 0,008) \times 10^4$ CFU/cm ³
TS № 27	$(1,2 \pm 0,006) \times 10^4$ CFU/cm ³	$(1,1 \pm 0,004) \times 10^4$ CFU/cm ³
TS № 28	$(9,0 \pm 0,045) \times 10^4$ CFU/cm ³	$(9,1 \pm 0,036) \times 10^4$ CFU/cm ³
TS № 29	$(3,7 \pm 0,018) \times 10^4$ CFU/cm ³	$(3,6 \pm 0,014) \times 10^4$ CFU/cm ³
TS № 30	$(2,1 \pm 0,010) \times 10^4$ CFU/cm ³	$(2,0 \pm 0,008) \times 10^4$ CFU/cm ³
TS № 31	$(1,0 \pm 0,005) \times 10^4$ CFU/cm ³	$(1,1 \pm 0,013) \times 10^4$ CFU/cm ³
TS № 32	$(1,1 \pm 0,051) \times 10^5$ CFU/cm ³	$(1,3 \pm 0,041) \times 10^5$ CFU/cm ³
TS № 33	$(1,5 \pm 0,076) \times 10^5$ CFU/cm ³	$(1,6 \pm 0,061) \times 10^5$ CFU/cm ³
TS № 34	$(3,9 \pm 0,019) \times 10^4$ CFU/cm ³	$(4,0 \pm 0,016) \times 10^4$ CFU/cm ³
TS № 35	$(8,9 \pm 0,044) \times 10^4$ CFU/cm ³	$(9,0 \pm 0,036) \times 10^4$ CFU/cm ³
TS № 36	$(3,7 \pm 0,018) \times 10^4$ CFU/cm ³	$(3,6 \pm 0,014) \times 10^4$ CFU/cm ³
TS № 37	$(8,6 \pm 0,043) \times 10^4$ CFU/cm ³	$(8,5 \pm 0,034) \times 10^4$ CFU/cm ³
TS № 38	$(1,3 \pm 0,006) \times 10^4$ CFU/cm ³	$(1,4 \pm 0,011) \times 10^4$ CFU/cm ³
TS № 39	$(9,1 \pm 0,045) \times 10^4$ CFU/cm ³	$(9,0 \pm 0,036) \times 10^4$ CFU/cm ³

$p < 0,05$

- for MAFAnM: the value obtained for each sample using the TEMPO® automated system and the reference method of plating on PCA agar.
- n – number of observations (sample size)

Section 2

t - Statistics for a paired t-test:

$$t = \frac{\bar{d}}{s_d / \sqrt{n}}$$

$t \approx 1,80$ somatic cells/cm³

$t \approx -1,32$ CFU/cm³ (MAFAnM)

where,

s_d - standard deviation of the differences

Critical value at $\alpha = 0,05$, $df = 38$:

$t_{cr} \approx 2,024$ somatic cells/cm³

$t_{cr} \approx 2,02$ CFU/cm³ (MAFAnM)

Comparison: $|t_{count}| = 1,80 < t_{cr} = 2,024$ somatic cells/cm³

Comparison: $|t_{count}| = 1,32 < t_{cr} = 2,02$ CFU/cm³³ (MAFAnM)

Thus, the mean values from the two somatic cell counts (301,795 and 294,641 cells/cm³) and the standard deviations (44,725 cells/cm³ and 42,462 cells/cm³) are similar, indicating comparable variability in the results. The calculated t-value ($t = 1.80$) at $df = 38$ is less than the critical value ($t_{cr} = 2.024$, $\alpha = 0.05$, two-tailed test). Thus, no statistically significant differences were found between the methods for determining somatic cell counts ($p > 0.05$). The methods are consistent and equivalent in terms of measurement results.

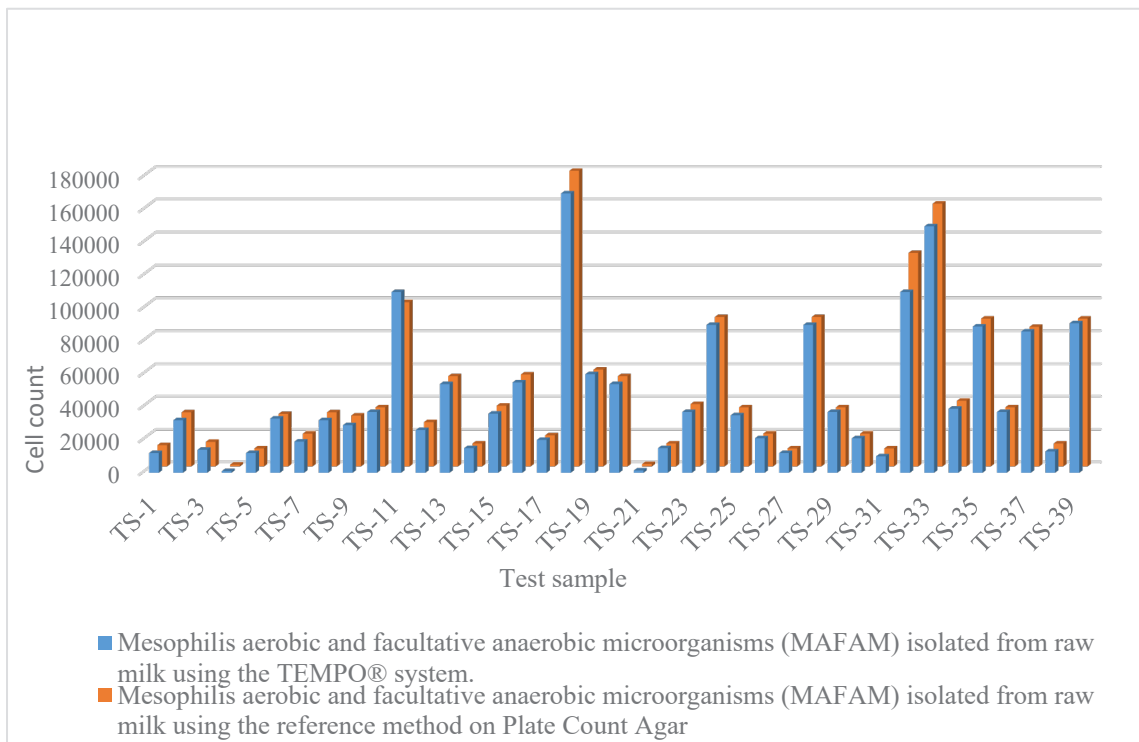


Fig. 8. Results of isolation and enumeration of MAFAM in raw milk using two research method

The mean values obtained from milk testing using the MAFAnM system via two different methods (43,041 CFU/cm³ and 43,513 CFU/cm³) and standard deviations (36,360 CFU/cm³³ and 36,630 CFU/cm³³) are practically identical, indicating a high degree of consistency in the results. The calculated t-value ($t = -1.32$) at $df = 38$ is less than the critical value $t_{cr} = 2.020$, $\alpha = 0.05$, two-tailed test). Thus, no statistically significant differences were found between the automated TEMPO® method and the reference method of inoculation on PCA agar ($p > 0.05$). The methods are statistically equivalent for the determination of MAFAnM. The analysis was based on the results of a study of 39 paired samples. The formula for Pearson's correlation coefficient:

$$r = \frac{\sum_{i=1}^n (x_i - \bar{x}) \times (y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 \times \sum_{i=1}^n (y_i - \bar{y})^2}}$$

$n = 39$

For 39 paired samples, the following results were obtained: $r = 1,0$ somatic cells/cm³

$r = 0.9996$ CFU/cm³ (MAFAnM)

Thus, the results of the correlation analysis show that there is a very strong direct linear relationship between the results of somatic cell counts obtained using different counting methods and the results of MAFAnM determination using the automated TEMPO® method and the reference method of plating on PCA agar. For somatic cells, the correlation coefficient is $r = 1.0$ —indicating perfect agreement of the results—while for MAFAnM, $r = 0.9996$, indicating virtually complete agreement between the methods. In both cases, the correlation is statistically significant ($p < 0.05$), which confirms the reliability of the results obtained and indicates the equivalence of the research methods used.

Following experimental analyses of 39 raw milk samples, they were classified by grade in accordance with the requirements of DSTU 3662:2018. The samples were classified based on quality indicators, specifically somatic cell count and the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM). The results obtained made it possible to assess the sanitary and hygienic condition of the milk and classify each sample into the appropriate grade in accordance with established regulatory criteria. The proportion of each grade (extra, premium, first, and ungraded) was determined as a percentage of the total number of samples tested (Figs. 9-10).

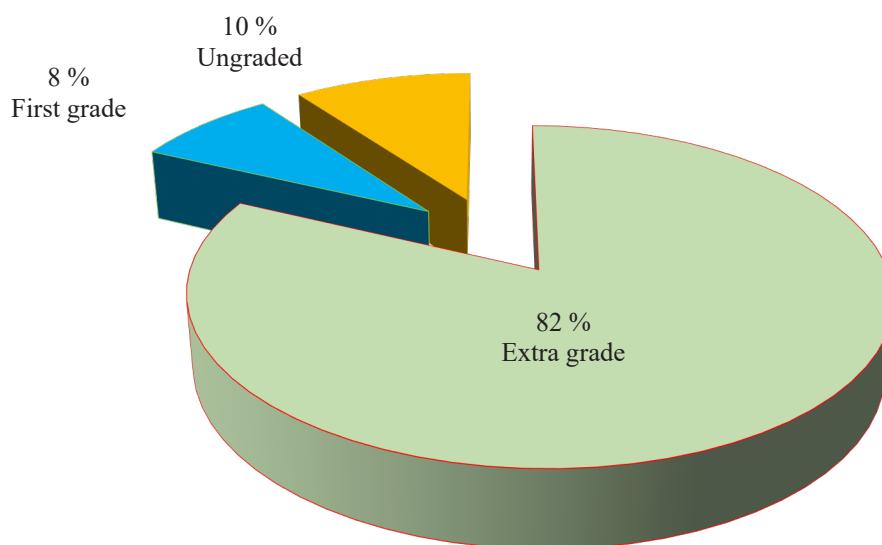


Fig. 9. Distribution of raw milk samples by grade based on somatic cell count, %

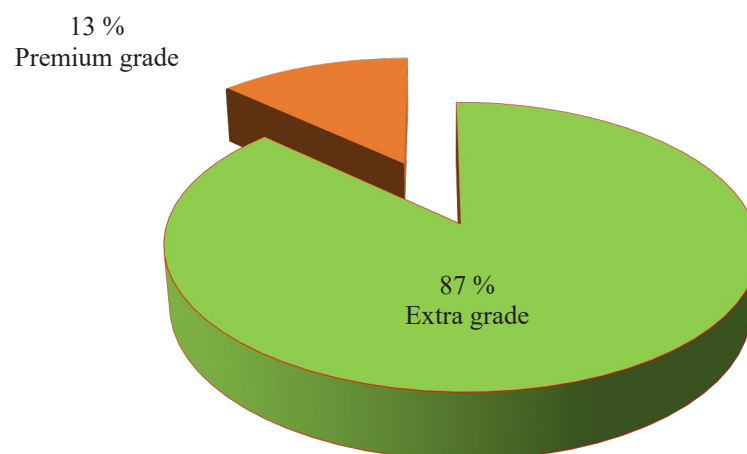


Fig. 10. Distribution of raw milk samples by grade based on the percentage of MAFAnM, %

Conclusions. The study analyzed 39 samples of raw cow's milk from clinically healthy Black-and-White and Red dairy cows aged 4–8 years, collected from private farms in various regions of Ukraine.

The determination of somatic cells in raw milk was performed using two microscopic methods with Newman–Lampert staining in accordance with ISO 13366-1:2008. Both methods allow for the quantitative assessment of epithelial cells and leukocytes, reflecting the physiological condition of the udder and milk quality. The results demonstrated the high accuracy and practical applicability of both approaches. Pearson's correlation analysis revealed a strong positive linear relationship between the results of the two methods, indicating their analytical equivalence. Student's paired t-test did not reveal any statistically significant differences between the mean values of the samples ($p > 0.05$), confirming the reliability, reproducibility, and stability of the results obtained by both methods.

An analysis of 39 raw milk samples showed that most of the milk met high-quality standards in terms of somatic cell count. Specifically, 82% of the samples met the requirements for extra-grade milk, 8% for first-grade milk, and 10% were classified as ungraded milk. The results indicate an overall adequate level of milk production hygiene; however, the presence of ungraded raw milk points to the need to improve animal housing conditions and strengthen health monitoring.

The testing of raw milk for the presence of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) was conducted using the TEMPO® automated system and conventional deep plating on Plate Count Agar in accordance with ISO 4833-1:2013. Statistical analysis revealed no significant differences between the results of the automated TEMPO® method and the reference plating method ($p > 0.05$). Correlation analysis showed a high positive correlation between the results of the two methods, with the MAFAnM correlation coefficient indicating virtually complete agreement ($p < 0.05$), confirming the reliability of the data obtained. Regression analysis demonstrated a proportional relationship between the quantitative indicators obtained by different methods, without any systematic bias in the results, indicating the absence of significant methodological error when using the automated TEMPO® system.

The results obtained using the TEMPO® system confirmed the feasibility of using the system as an alternative method for determining MAFAnM in raw milk in production laboratory settings. The TEMPO® system ensured the reliability, accuracy, and reproducibility of results comparable to those of the classical culture method. The TEMPO® system demonstrated significant advantages, including reduced analysis time, ease of use, and minimized human factor.

The results of the assessment of raw milk for MAFAnM content showed that 87% of the samples met the “Extra” grade standards, while 13% met the “Superior” grade standards, indicating a high overall quality of the raw material tested.

The low somatic cell counts in the samples analyzed fell within the normal range for physiologically healthy milk, confirming the absence of inflammatory processes in the udder and the animals' good health. The MAFAnM levels varied depending on housing conditions, animal care, and milking techniques. Low MAFAnM values indicated high milk hygiene, while elevated values may indicate hygiene violations or issues with the processing technology. Regular monitoring of somatic cells and MAFAnM is an effective tool for assessing the physiological condition of the udder, the sanitary quality of milk, and its technological suitability, which is of direct importance for ensuring the safety and nutritional value of dairy products.

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Комплексна оцінка стану сирого коров'ячого молока за вмістом соматичних клітин і мезофільних аеробних та факультативно-анаеробних мікроорганізмів як індикаторів безпечності та технологічних властивостей молочної сировини

Тогачинська Л. (ORCID: 0009-0005-5032-5940), Курята Н. (ORCID: 0000-0002-6958-1064), Мусієць І. (ORCID: 0000-0002-2456-560X), Піщанський О. (ORCID: 0009-0002-0111-4977), Галка І. (ORCID: 0000-0001-8701-3506), Баланчук Л. (ORCID: 0000-0003-0989-5886), Куликова В. (ORCID:0009-0008-8827-030X)

Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи, м. Київ, Україна, e-mail: toq.liya888@gmail.com

Резюме. У роботі проведено порівняльну оцінку методів визначення вмісту соматичних клітин та кількості мезофільних аеробних і факультативно-анаеробних мікроорганізмів (МАФАНМ) у сирому коров'ячому молоці. Метою дослідження було встановлення аналітичної узгодженості, точності та відтворюваності результатів, отриманих різними методами, а також обґрунтування можливості застосування автоматизованої системи TEMPO® як альтернативного методу мікробіологічного контролю.

Матеріалом дослідження слугували 39 зразків нативного коров'ячого молока, відібраних у 2025 році від клінічно здорових корів чорно-рябої та червоної молочних порід віком 4–8 років у приватних господарствах різних регіонів України. Визначення кількості соматичних клітин здійснювали двома мікроскопічними методами відповідно до вимог ISO 13366-1:2008: шляхом підрахунку клітин у мазках у п'ятдесяти полях зору та у

десяти паралельних смугах. Кількість МАФАНМ визначали двома незалежними методами: автоматизованим флуориметричним методом із використанням системи TEMPO® та еталонним методом глибинного посіву на поживне середовище Plate Count Agar згідно з ISO 4833-1:2013.

Статистичну обробку результатів здійснювали із застосуванням парного *t*-критерію Стьюдента та кореляційного аналізу Пірсона. Встановлено, що середні значення показників соматичних клітин, отримані різними методами, становили 301795 та 294641 клітин/см³ відповідно, а для МАФАНМ — 43041 та 43513 КУО/см³. Розраховані значення *t*-критерію ($t = 1,80$ для соматичних клітин та $t = -1,32$ для МАФАНМ) не перевищували критичних значень ($p > 0,05$), що свідчить про відсутність статистично значущих відмінностей між методами. Коефіцієнт кореляції Пірсона становив $r = 1,0$ для соматичних клітин та $r = 0,9996$ для МАФАНМ ($p < 0,05$), що вказує на наявність тісного прямого лінійного зв'язку та високу узгодженість результатів.

Дослідження показали, що автоматизована система TEMPO® забезпечує результати визначення мезофільних аеробних та факультативно-анаеробних мікроорганізмів (МАФАНМ) у сирому молоці, статистично еквівалентні еталонному методу, з високою точністю та відтворюваністю і мінімальним впливом людського фактору. Використання цієї системи доцільне для впровадження у виробничих лабораторіях як оперативного та надійного інструменту контролю мікробіологічних показників молочної сировини.

Ключові слова: соматичні клітини, мезофільних аеробних і факультативно-анаеробних мікроорганізмів (МАФАНМ), молоко коров'яче, ґатунок, автоматизований метод TEMPO®, кореляційний аналіз, *t*-критерій Стьюдента

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