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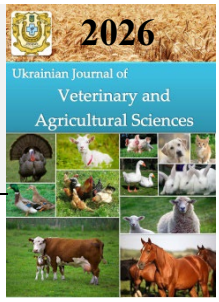

Modern diagnostic tools for subclinical mastitis in cows

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Abstract

After Ukraine joined the World Trade Organization, requirements for the safety and quality of food products, particularly milk and its processed products, have significantly increased in both domestic and international markets. Milk remains one of the basic products in the population's diet. However, its nutritional value and health-promoting properties are preserved only if a high level of sanitary quality is maintained and the indicators fully comply with current state standards. An important factor in ensuring the quality of dairy raw materials is early diagnosis of subclinical mastitis, which is not accompanied by pronounced clinical signs but significantly affects milk composition and safety. Therefore, the work aimed to detect the latent form of subclinical mastitis promptly using the most modern and effective method for determining somatic cells, in a comparative context. Veterinary and sanitary examination of milk ensures control of product quality and safety before sale. The main studies included organoleptic evaluation, physicochemical parameters, and determination of somatic cell content, a key marker of udder health and milk quality. The article presents the results of research on the quality of raw milk sold in the agro-industrial markets of Mykolaiv. The Californian mastitis test, De Laval, and the Mastydine R test demonstrated the same effectiveness in detecting subclinical mastitis, with a positive reaction in 8 samples (16%) out of 50 studied. The milk settling test showed slightly lower efficiency, with a positive reaction in 6 samples (12 %) out of 50 tested. Therefore, the California test and the Mastidine R test are the most appropriate, and the settling test yielded less accurate results. Milk with a positive reaction is prohibited for free sale. Thus, determining the content of somatic cells is a mandatory element of the veterinary and sanitary assessment of milk on the markets and plays an important role in ensuring the safety and quality of dairy raw materials in accordance with current national standards.

Keywords: raw milk; somatic cells; subclinical mastitis; settling test; Californian De Laval test; Mastidine R test; organoleptic indicators; physicochemical indicators.

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1. Introduction

Dairy farming is the leading livestock industry, providing the country's population with milk. Various animal diseases hinder successful herd reproduction and increased productivity (Kasiianchuk et al., 2015).

One of the most common problems in dairy farming in veterinary medicine is mammary gland disease – cow mastitis. The global cow population is 211 million animals, of which over 40% suffer from various forms of mastitis (Suprovych & Stroianovska, 2023).

Mastitis is an inflammation of the mammary gland caused by various damaging factors and characterized by pathological changes in its tissues and secretions. Symptoms include fever, tachycardia, gastrointestinal damage, and hypogalactia. One type of mastitis is subclinical (latent) mastitis (mastitis latentus). This disease is characterized by a subacute inflammatory process with virtually no clinical signs, but includes hypogalactia and changes in the physicochemical properties and cellular composition of milk. Subclinical mastitis is a long-term condition that reduces milk production, degrades milk quality, and leads to premature culling of dairy cattle. Subclinical mastitis is considered to be the most damaging to dairy farms (Tommasoni et al., 2023; Stoichescu et al., 2026).

Mastitis is no exception in our country, with 3–50 % of animals affected, and 70 % of cases are subclinical.

The etiology of mastitis varies widely, but microorganisms – bacteria, fungi, and viruses – present in cows' milk play a major role. Cows with mastitis may have reduced milk quality, which can lead to illness not only in young animals but also in humans (Safak & Risvanli, 2021; Suprovych et al., 2025).

Milk for consumers must be of high quality, high in nutritional value, and contain sufficient fat, protein, minerals, and vitamins. Separate requirements pertain to safety: safe milk must not contain pathogenic bacteria, antibiotics, or sulfa drugs.

Therefore, according to the "Machine Milking Rules" and veterinary legislation, the entire dairy herd must be tested at least once a month under farm conditions (Ibatullin et al., 2025).

With WTO membership, Ukraine has increased its responsibility for maintaining the high quality of its products, particularly milk and dairy products, in both domestic and foreign markets. Milk must be of high sanitary quality and meet state standards. Safe milk can only be obtained from healthy animals that meet all necessary sanitary, hygienic, and veterinary requirements at all stages of production (Kasiianchuk et al., 2015; Suprovych et al., 2025; Suprovych & Stroianovska, 2023; Phiphattanaphiphop et al., 2026).

The economic impact of mastitis in cows stems from deteriorating milk quality. The delayed diagnosis and identification of cows with subclinical mastitis particularly impacts this. Therefore, in recent years, intensive scientific development of methods for diagnosing, preventing, and treating subclinical mastitis has become especially important (Ibatullin et al., 2025; Nagy et al., 2025).

Research has encompassed both traditional methods and several promising electronic devices. As a result of these advances, specialized diagnostic methods have been developed that effectively detect both clinical and subclinical mastitis in cows, enabling early diagnosis and improving the effectiveness of preventive measures in dairy farming (Peckler & Adcock, 2025; Zeweld & Tarekegn, 2025; Jember et al., 2026).

Milk is an essential component of human nutrition. However, it also serves as a favorable environment for the growth of microorganisms, particularly dangerous pathogens (*Salmonella spp., E. coli, Listeria monocytogenes, Staphylococcus aureus*, etc.). One of the main sources of bacterial contamination of milk is mastitis in cows, particularly its subclinical form, which often remains undetected and significantly impairs product quality. Ensuring the sanitary quality of milk requires timely mastitis diagnosis, adherence to milking hygiene, proper animal welfare, and compliance with veterinary and sanitary requirements.

According to Kotelevych V. A. et al. (2025), mastitis is one of the leading pathologies in dairy farming, leading to a deterioration in the quality and safety of dairy products and posing a significant threat to consumer health. This research team reports that mammary gland pathologies, primarily inflammatory ones, remain among the leading factors in reduced productivity and deterioration in the sanitary quality of milk. Among these, mastitis in dairy cows, particularly its subclinical form, is a pressing problem both in Ukraine and internationally (Kotelevych et al., 2025).

Inflammatory lesions of the mammary gland significantly affect the quality of raw milk, altering its chemical composition and physical and biological characteristics. As a result, the milk loses nutritional value and becomes unsuitable for further technological processing (Kasiianchuk et al., 2015; Suprovych & Stroianovska, 2023; Suprovych et al., 2025).

According to veterinary and sanitary inspection requirements, milk obtained from cows with mastitis is not permitted for consumption or sale, as it contains increased levels of pathogenic microorganisms and toxins and is characterized by increased acidity. These properties make it impossible to use this raw material for the production of fermented milk products, particularly cheeses. Microbiological activity in milk degrades proteins, lipids, and other nutritional components, thereby negatively affecting the organoleptic properties of finished products. Furthermore, milk may contain heat-resistant toxins that are not inactivated by heat treatment (Riekerink et al., 2007; Moradi et al., 2021).

The authors report that cows with mastitis experience a significant deterioration in the sanitary and physicochemical parameters of milk. In the subclinical form of mastitis, the somatic cell count reaches 1,028.4 thousand/cm³, and in the clinical form, 420.2 thousand/cm³, whereas in healthy animals it does not exceed 119.3 thousand/cm³. At the same time, bacterial contamination of milk increases sharply – from 120.0 to 3981.2 thousand/cm³. Milk acidity decreases by 0.7 °T in subclinical and by 0.4 °T in clinical mastitis compared to healthy cows. Deterioration in the sanitary quality and physical properties of milk in various forms of mastitis leads to a decrease in its grade and subsequent culling (Carvalho-Sombra et al., 2021; Radzykhivskyi et al., 2023).

Milk production is an important sector of Ukrainian agriculture, and its effectiveness depends on cow health. Mastitis is one of the most common and costly diseases, reducing milk production and impairing the quality and safety of milk. Subclinical mastitis, which is asymptomatic and can go undetected, is particularly dangerous; it is detected by measuring the somatic cell count in milk (Kovalenko, 2022; Shrestha et al., 2023).

Mammary gland diseases cause significant economic losses in dairy farming, with mastitis being the leading cause. The incidence of the disease varies from 3% to 50% among lactating cows on different farms, with only approximately 30% of cases having clinical manifestations, while the vast majority are subclinical. Early diagnosis of subclinical mastitis is of significant economic, sanitary, and technological importance, as it determines the effectiveness of timely treatment, prevention of clinical disease, and milk quality assurance (Kasiianchuk et al., 2015; Suprovych & Stroianovska, 2023; Suprovych et al., 2025; Koreiba et al., 2025).

According to Koreyba L. V. et al. (2025), despite prevention and treatment, mastitis remains the most common health problem in cows in Ukraine, leading to a decrease in milk and dairy product production and negatively impacting animal health, welfare, and the economy of the livestock industry (Ordin et al., 2024; Koreiba et al., 2025).

Subclinical mastitis at the beginning and peak of lactation negatively impacts cow productivity, leading to decreased milk yield and deterioration in milk quality, and increases the risk of pathogen spread. Biochemical, cytological, and bacteriological methods are used for its diagnosis. Biochemical approaches involve measuring changes in milk pH and, indirectly, detecting elevated somatic cell counts using rapid tests. Cytological methods are based on somatic cell counts and are highly accurate, but require specialized equipment. Bacteriological studies allow identification of the pathogen and selection of etiotropic therapy, although their informativeness may be reduced by changes in the microflora or the amicable nature of the inflammation. During the colostrum period, an enzyme-linked immunosorbent assay (ELISA) is advisable for early detection of subclinical mastitis, enabling diagnosis as early as 5 days after calving (Anueyiagu et al., 2022; Ribeiro et al., 2023).

The causes of mastitis are conventionally classified into several main groups. Physical factors include adverse microclimatic conditions, particularly cold, high temperatures, drafts, and excessive humidity, as well as mechanical damage to the udder – bruises, contusions, wounds, skin cracks, and microtraumas, often resulting from improper milking techniques. Chemical factors are associated with the action of aggressive substances, particularly acids, alkalis, gases, phytoestrogens, and various toxins. Biological causes are due to the influence of pathogenic microorganisms, among which the most common are *Streptococcus* (*Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis*), *Staphylococcus* (including *Staphylococcus aureus*, which produces alpha-hemolysin), and mycoplasmas. These pathogens differ in both their pathogenic properties and environmental characteristics. A separate group consists of endogenous causes associated with disruptions in the functioning of the body's biological systems, in particular, with a decrease in the protective properties of the mucous membranes, the development of allergic reactions, and neurodegenerative processes.

Several researchers note that mastitis and infections are a serious health problem for cattle. Factors contributing to the development of mastitis include the introduction of pathogens into the udder by calves through suckling, unfavorable housing conditions such as wet and dirty bedding, or insect infestation. The disease is often asymptomatic, making diagnosis difficult. It results in decreased milk production. Treatment primarily involves antibiotics, but many asymptomatic cases remain untreated, underscoring the need for early and timely diagnosis (Holumbiiovska et al., 2024).

According to the research of Perkiy Yu. B. and coauthors, preventive anti-mastitis measures on dairy farms are of fundamental importance for maintaining the milk productivity of the herd and, above all, in the diagnosis of mastitis in heifers at 8–9 months of pregnancy and in its treatment before birth (Perkii et al., 2023).

Mastitis remains one of the most common and economically significant pathologies in dairy farming, which makes it possible to prevent subclinical cases. Subclinical mastitis negatively affects milk yield and quality, delays the onset of the disease, and facilitates the spread of pathogens within the herd. At the same time, milk as a food product must meet established quality and safety requirements, making mastitis control particularly necessary. Effective mastitis control begins with early diagnosis using organoleptic, physicochemical, cytological, bacteriological, and sanitary methods, as well as comprehensive preventive measures, including examinations and animal care during the fertile period. Implementing these approaches is key to maintaining cow health, ensuring high-quality and safe milk, and increasing the productivity and economic efficiency of dairy production (Riekerink et al., 2007; Kasiianchuk et al., 2015; Carvalho-Sombra et al., 2021; Moradi et al., 2021; Radzykhovskyi et al., 2023; Suprovych & Stroianovska, 2023; Perkii et al., 2023; Holumbiiovska et al., 2024; Suprovych et al., 2025; Ibatullin et al., 2025; Olishevskyi & Huralska, 2025; Kotelevych et al., 2025; Wang et al., 2026).

The work aims to study the timely detection of the latent form of subclinical mastitis using the most modern effective method for determining somatic cells in a comparative aspect.

Research objectives:

- to examine cow's milk using organoleptic methods;
- to determine the physicochemical properties of cow's milk;
- to establish the most effective rapid method for comparative determination of somatic cells.

2. Materials and methods

The study's material consisted of 50 samples of raw cow's milk, collected during its sale at agro-food markets in the city of Mykolaiv in 2025. Samples were collected randomly from batches of milk from different suppliers in standard sterile containers.

Milk samples were analyzed using the EKOMILK milk analyzer. This device determines the parameters of milk and other processed products and monitors the parameters of formula milk. Modern rapid methods were also used to detect subclinical mastitis. Therefore, raw milk quality control was based on a combination of parameters, including organoleptic, physicochemical, and sanitary characteristics, including somatic cell count.

Organoleptic evaluation of milk is a mandatory step in veterinary and sanitary inspections, enabling rapid assessment of its quality and freshness without specialized laboratory equipment. These indicators reflect the sanitary condition of the product, its production, storage, and transportation conditions, and also allow for the detection of initial signs of spoilage (Ibatullin et al., 2025).

All experimental studies were conducted in accordance with the “General Ethical Principles of Animal Experiments” (Ukraine, 2001), in compliance with the Law of Ukraine “On the Protection of Animals from Cruel Treatment,” as well as the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985).

3. Results and discussion

In 42 samples, the milk exhibited a homogeneous structure, no clots, and a smooth, fluid consistency. The color was white or slightly creamy. The taste was pleasant and slightly sweet. The odor was characteristic of fresh milk, with no foreign odors detected. However, in 8 samples, flakes and mucus were observed; the milk had a pinkish tint and a slightly sour odor, indicating the initial stages of spoilage.

Physicochemical parameters are among the key criteria used to assess the quality and authenticity of raw milk during veterinary and sanitary inspection. They enable compliance with regulatory requirements, detection of adulteration, and evaluation of technological suitability. These parameters include titratable acidity, density, fat content, protein content, and solids-not-fat (SNF) content (Kasiianchuk et al., 2015; Suprovych & Stroianovska, 2023; Suprovych et al., 2025; Ibatullin et al., 2025; Kotelevych et al., 2025).

The study revealed that 42 milk samples had physicochemical parameters within the normal range, whereas 8 samples exhibited abnormalities. In particular, the titratable acidity was reduced to 10–13°T (Fig. 1). This may indicate an increased alkaline reaction associated with pathological changes in the mammary gland.

In 42 raw milk samples, the density at 20°C ranged from 1.027 to 1.032 g/cm³. In three samples, the density was reduced to 1.019–1.020 g/cm³, suggesting dilution with water. In contrast, five samples showed a density of 1.033–1.0345 g/cm³, suggesting partial skimming of the milk (Fig. 2).

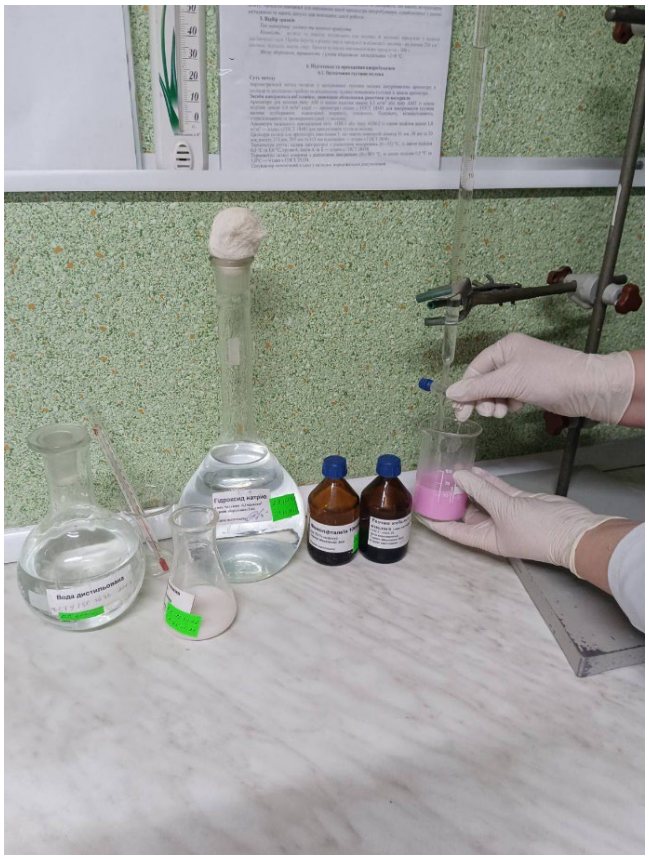

Fig. 1. Determination of milk acidity

Fig. 2. Determining milk density

Milk fat, one of the principal components of milk, largely determines its nutritional value and sensory characteristics. The fat content of raw cow's milk in 42 samples ranged from 3.2 % to 4.0 %. In three samples, the fat content ranged from 3.0 % to 3.1 %, whereas in five samples it ranged from 4.2 % to 4.5 %, suggesting adulteration through partial skimming or manipulation of milk composition.

Milk proteins, particularly casein, have a high biological value and play a crucial role in determining milk's technological properties. In 42 samples, the protein content ranged from 2.8 % to 3.4 %, which is within the normal range. In three samples, the protein content was reduced to 2.2–2.5 %, which may be attributed to dilution with water. In five samples, the protein content ranged from 3.5 % to 3.7 %.

Solids-not-fat (SNF) include proteins, lactose, and mineral substances and represent a stable indicator of milk authenticity. In 42 samples, the SNF content ranged from 8.2 % to 8.4 %. In three samples, this value was lower (8.0–8.1 %), indicating possible dilution with water.

Based on the physicochemical analyses, 8 milk samples were identified as adulterated, indicating unsatisfactory product quality.

Timely detection of subclinical mastitis is an important component of veterinary and sanitary control of milk quality, as inflammatory processes in the mammary gland significantly affect the physicochemical, organoleptic, and microbiological characteristics of milk. Rapid diagnostic methods based on elevated somatic cell counts are among the most widely used approaches in veterinary medicine and veterinary-sanitary inspection. These methods include the Mastidine reagent test, the DeLaval California Mastitis Test (CMT), and the sedimentation test (Narváez-Semanate et al., 2022; Radzykhovskyi et al., 2023).

Somatic cell determination was performed using a comparative approach involving the Mastidine R test, the DeLaval California Mastitis Test (CMT), and the sedimentation test.

The Mastidine R test is based on the interaction of the reagent with the nucleic acids of somatic cells, resulting in the formation of a gel-like mass of varying viscosity. The degree of milk thickening and the consistency of the resulting gel correlate with the somatic cell count. This method is highly sensitive, simple to perform, and suitable for on-farm use. Its main advantage is the ability to detect subclinical forms of mastitis; however, the results are semi-quantitative and therefore require laboratory confirmation (Kovalenko, 2022; Radzykhovskyi et al., 2023).

The DeLaval California Mastitis Test (CMT) is one of the most widely used rapid diagnostic methods for mastitis worldwide. Similar to the Mastidine R test, it is based on the reaction between a detergent reagent and somatic cells in milk, which results in gel formation. The results are visually evaluated based on the reaction intensity. Compared with the Mastidine R test, the DeLaval CMT offers greater reproducibility and a more standardized interpretation of results, which enhances its diagnostic value under commercial dairy production conditions (Kovalenko, 2022; Radzykhovskyi et al., 2023; Hisira et al., 2023).

The sedimentation test is a simple method for assessing milk quality and is based on visual evaluation of changes that occur during storage at low temperatures for 12–24 h. In

milk obtained from cows with mastitis, clots, flakes, sediment, or a watery layer may develop during storage. Unlike chemical diagnostic tests, the sedimentation test has relatively low sensitivity and is primarily suitable for detecting clinical or advanced forms of mastitis. Nevertheless, its advantage is that it does not require reagents or specialized equipment (Kovalenko, 2022; Radzykhovskyi et al., 2023).

Thus, according to the available literature, a comparative analysis indicates that the Mastidine R test and the DeLaval California Mastitis Test (CMT) are more informative for early mastitis diagnosis, as both methods detect elevated somatic cell counts in milk. The DeLaval CMT is characterized by greater standardization, whereas the Mastidine R test is more widely available in domestic veterinary practice. The sedimentation test may be used as a preliminary method for assessing milk quality; however, it lacks sufficient sensitivity to detect subclinical mastitis.

The results of somatic cell determination in cow's milk were evaluated by visual assessment of the milk reaction following the addition of the respective reagents, with the intensity of changes in consistency and structure taken into account. During rapid diagnostic testing, particular attention was paid to the degree of thickening, gel formation, the presence of flakes or sediment, and changes in whey clarity, which directly or indirectly reflected the somatic cell content.

The results of the Mastidine R test and the DeLaval California Mastitis Test were interpreted using a grading scale from negative to strongly positive. The intensity of the reaction correlates with the increase in somatic cell count and serves as a preliminary indicator of the severity of inflammatory processes in the mammary gland.

The sedimentation test was evaluated based on the presence or absence of visible changes in milk after storage, including sediment formation, the quantity and characteristics of the separated milk fraction, and milk color. Milk obtained from healthy cows is typically white or slightly bluish and does not form sediment during storage. In contrast, milk from cows affected by mastitis often appears watery, while the separated fraction may become viscous, mucous, or contain flakes.

The obtained results were interpreted in accordance with the manufacturers' instructions for the diagnostic tests used.

The correspondence between the reaction scores of the rapid diagnostic methods and the somatic cell count levels is presented in Table 1.

Table 1
Correspondence of rapid test reaction scores to the somatic cell count

Reaction score	Milk reaction pattern (De Laval California Mastitis Test, Mastidine R)	Veterinary and sanitary assessment
0 (–)	Homogeneous liquid, no signs of thickening. Light lilac color	Milk from clinically healthy cows
1 (+)	Weak thickening, unstable small flakes. The color of the mixture ranges from light lilac to lilac	Acceptable initial changes
2 (++)	Marked thickening, formation of dark lilac gel	Subclinical mastitis
3 (+++)	Sharp thickening, dense gel, lumps	Clinical mastitis, milk is unsuitable

The results showed a relationship between reaction intensity and the quality of the raw milk. Changes in milk consistency observed during the DeLaval California Mastitis Test (CMT) and the Mastidine R test were associated with increased somatic cell concentrations and their lysis products. This enables these methods to be used as rapid screening tools for estimating somatic cell counts and detecting subclinical mastitis.

According to the somatic cell count results, 42 milk samples complied with veterinary and sanitary requirements. In contrast, 8 samples exhibited milk thickening and gel formation, corresponding to a score of 2 (++), which is indicative of subclinical mastitis.

Table 2 presents the results of rapid diagnostic test detection of subclinical mastitis.

Analysis of the results presented in Table 2 revealed that the DeLaval California Mastitis Test (CMT) and the Mastidine R test demonstrated identical diagnostic performance in detecting subclinical mastitis, yielding positive results in 8 of the 50 milk samples examined (16.0 %). In contrast, the sedimentation test showed slightly lower diagnostic sensitivity, with positive results recorded in 6 of the 50 samples tested (12.0 %).

Thus, the evaluation of somatic cell determination results was based on an integrated approach, which ensured the objectivity and reproducibility of the data obtained.

Table 2
Results of subclinical mastitis diagnostics using rapid tests

Methods for diagnosing subclinical mastitis	Research results		
	Samples	positive test	negative test
California Milk Test DeLaval	50	8	42
Mastidin P Assay	50	8	42
Milk Settling Test	50	6	44

Fig. 3. Samples with Mastidine R test

Fig. 4. DeLaval California Test

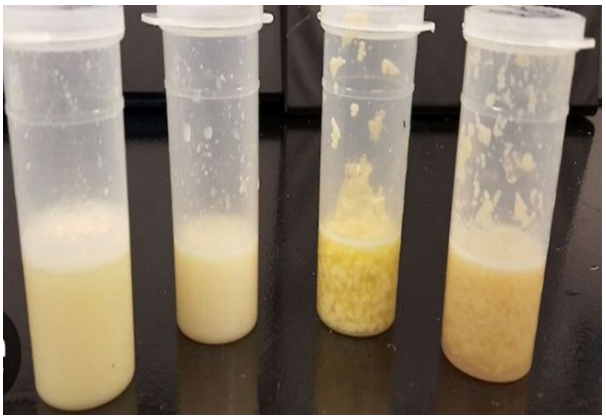

Fig. 5. Sedimentation test

4. Conclusions

According to the organoleptic indicators reflecting the sanitary condition of the product, 42 out of 50 raw cow's milk samples complied with veterinary and sanitary requirements. However, eight samples contained flakes and mucus, exhibited a slightly pinkish color, and had a sour odor, indicating the initial stages of spoilage.

Based on the physicochemical assessment, 42 milk samples met the applicable DSTU standards, whereas 8 samples exhibited deviations indicative of milk adulteration. These deviations included dilution with water, partial skimming, and reduced titratable acidity accompanied by a shift toward an alkaline reaction, suggesting an increased content of alkaline components.

Among rapid diagnostic methods for detecting elevated somatic cell counts associated with subclinical mastitis, the DeLaval California Mastitis Test (CMT) and the Mastidine R test demonstrated the highest diagnostic effectiveness. Both methods identified positive reactions in 8 of the 50 milk samples examined (16.0%). In contrast, the sedimentation test showed lower diagnostic sensitivity, yielding positive results in 6 of the 50 samples (12.0%). Milk samples that tested positive for subclinical mastitis did not comply with veterinary and sanitary requirements for marketing and human consumption.

The results obtained confirm that somatic cell count determination is an essential component of the veterinary and sanitary assessment of milk marketed for human consumption and plays an important role in ensuring the safety and quality of raw milk in accordance with current national regulations.

Prospects for further research include monitoring the functional state of the mammary gland throughout lactation, developing preventive measures against various forms of mastitis, and conducting monthly screening of milk samples using rapid diagnostic tests to detect inflammatory processes in the mammary gland early.

Conflict of interest

The authors declare no conflict of interest.

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