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# LABORATORY STUDIES TO ASSESS THE DEGREE OF CONTAMINATION IN MILK

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**Annotation**
The article analyzes the methods of general bacterial contamination of milk, which are used in assessing the quality of milk raw materials.

**Keywords:** dairy farms, contamination, milk, microorganisms, udder, reductase test.

Milk from healthy cows is an important food product and raw material widely used in the food industry. Milk must be carefully monitored for all main indicators: organoleptic, physicochemical, microbiological and others, not only to determine its biological usefulness and safety, but also to determine its raw material value [1].

Currently, processing enterprises place increased demands on the quality of milk. To produce the full variety of dairy products, milk with high technological parameters is required. The main attention is paid to the sanitary and hygienic indicators of milk, which are primarily determined by technological factors [2].

Raw milk production in Uzbekistan in 2022 reached 11.6 million tons, 3% more than in 2021 and 29% more than in 2015. 93.4% of milk is produced in dekhan farms, 5.4% in farms, 1.2% in agricultural enterprises. The volume of production in dekhan farms in 2022 amounted to 10.8 million tons, 3% more than in 2021. Farms produced 632.2 thousand tons in 2022, 8% more than in 2021. Agricultural enterprises produced 135.8 thousand tons, 25% more than in 2021 [3].

Modern dairy plants or factories carry out complex processing of raw materials, produce a wide range of products, are equipped with mechanized and automated lines for bottling products into bottles, bags and other types of containers, pasteurizers and coolers, separators, evaporation units, cheese producers, and automatic product packaging machines [4] .

High bacterial contamination of milk significantly impairs its taste and also reduces its shelf life. Milk itself does not contain harmful microorganisms; their content in milk directly depends on compliance with veterinary and sanitary standards in livestock farming, since they enter the milk from contaminated udders, equipment and implements, air, water or personnel.

Bacterial contamination of milk is particularly increased with mastitis, since the number of somatic cells such as leukocytes and epithelial cells increases in the mouth of the udder.

Microbial contamination of milk can be caused in the following ways: the state of health of the animal itself, the content of pathogenic bacteria in feed and feed additives, water and pastures, as well as due to poor zoohygienic and veterinary sanitary measures.

Maintaining proper hygiene in keeping cows is an important fact to reduce the possibility of illness and, as a result, contamination of production facilities. Poor veterinary and sanitary treatment of premises and equipment can lead to microbial contamination of milk and the spread of pathogens. Udder contamination can occur during milking through contact between cows, staff's hands and milking equipment. The microbiological composition of raw milk is influenced by microorganisms present in the teat canal and the surface of the skin of the teat and udder. Milk contamination is greatly influenced by poor hygiene and disinfection of technological equipment, ambient air pollution in the milking parlor, as well as other zootechnical and animal hygiene factors. Another possibility of microbial contamination of milk may arise during long-term storage at insufficiently low temperatures [5].

Microbial contamination may also be affected by lack of storage conditions prior to processing.

Bacterial contamination can be determined in the following ways:

• direct counting of microorganisms
• titration inoculation or plate method
• reductase breakdown

Direct counting of microorganisms is easy to use, but not accurate enough, since when counting you cannot particularly distinguish living microorganisms from mechanical contaminants and somatic cells that collect in lumps.

In the plate method, we can only count the number of living microorganisms. For this reason, the titration sowing method is not accurate, since when sowing, MOs that no longer reproduce will not grow, anaerobic microorganisms will not grow, since sowing is carried out under aerobic conditions, it will be impossible to calculate the amount of mold and fungi, since their detection is possible only for 3-4 days.

“The microbial number is the number of bacterial colonies that grow on meat peptone agar when 1 ml of sample is inoculated and cultivated at 37°C for 24-48 hours (or 72 hours at 22°C).” Thus, the microbial number will be much smaller and will directly reflect only the number of saprophytic mesophilic aerobic microbes, which are the main causative agents of food spoilage. And it is by the microbial number that one can draw conclusions about the sanitary condition of the soil, water, air and food products that the animal consumes [6].

The most effective method is the reductase test method. This method is faster, as it takes an hour and a half, and it is also more effective than the counting method, since it relies on data obtained from the amount of reductase secreted , which is the subject of the vital activity of living organisms. “ Reductase is a waste product of bacteria. Since it is not present in freshly milked milk, it is necessary to create favorable conditions for its appearance. To do this, take a milk sample and heat it to a temperature of 36-38°C. Then this milk is mixed with resazurin dye and placed in a thermostat for 1-1.5 hours.



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Reductase tends to discolor the dye. So, over time, any colored milk should turn pale, but milk with few bacteria will retain its color longer. Thus, the highest and first grade milk, even after an hour and a half in the thermostat, will remain lilac in color, and low-grade milk will return a white tint within an hour.

| Duration of discoloration | Number of bacteria in 1 ml of milk | Milk class | Milk quality assessment |
|---|---|---|---|
| Over 5 hours 30 minutes | Less than 500 thousand | I | Good |
| From 2 o'clock to 5 o'clock 30 min | From 500 thousand to 4 million | II | Satisfactory |
| Over 20 min. Up to 2 hours | From 4 million to 20 million | III | Bad |
| 20 minutes or less | 20 million and above | IV | Very bad |

Thus, if the color does not change after 1 hour, the milk is recognized as the second grade, and if it continues to remain for another 30 minutes, it becomes the first or highest.

The main advantages of assessing the level of bacterial contamination of milk with a reductase test:

• the method is extremely simple to implement and does not require special equipment;
• low-cost , not requiring expensive reagents;
• proven by many years of experience in industry [7].

In order to check the quality of milk in laboratory conditions, we carried out a number of studies, one of which is the reductase test. For the study, we took a milk sample from the Mahmudjon dairy farm ota " and performed a reductase test. To do this, we took clean test tubes and poured 10 ml of milk and 0.5 ml of methylene blue, mixed and closed with a cotton stopper. Our solution of milk and methylene blue was placed in a thermostat at a temperature of 37-40˚C. We observed our solution until it completely discolored, because it is the time of completion of the reaction that indicates the degree of its bacterial contamination and classifies the milk into one class or another, but it should be borne in mind that the small colored part at the bottom of the test tube is not taken into account.

Based on the results of our work to assess the level of bacterial contamination of milk with reductase The sample determined that the milk belonged to the first class, since the duration of storage was more than an hour, and complete discoloration occurred in 3.5 hours. This says that in 1 cm $^3$ of milk there is approximately an amount of bacteria from approximately 300 thousand to 500 thousand, which classifies it as first class.

Consequently, of all the methods listed above, for determining bacterial contamination, the simplest to perform, least expensive and proven by many years of research is the reductase test reaction. And our studied milk from a dairy farm, according to the results of assessing the quality of milk for bacterial contamination with a redutase test, belongs to the first grade, which makes it safe.



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