



TRAICON  
Publishing  
House

SCIENTIFIC ACHIEVEMENTS IN ENVIROMENTAL AND LIFE  
SCIENCE

ISBN 978-83-65180-20-9  
2018, pp 6-19

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## IMPROVEMENT STRATEGIES FOR HORIZONTAL METHODS OF BACTERIOLOGICAL STUDY OF MILK

Tetiana PRYLIPKO<sup>1</sup>, Natalia BUKALOVA<sup>2</sup>, Nadiya BOGATKO<sup>2</sup>

<sup>1</sup>STATE AGRARIAN AND ENGINEERINGUNIVERSITY IN PODILYA, UKRAINE

<sup>2</sup>UNIVERSITY IN BILA TSEKVA, UKRAINE

\*Corresponding author: e-mail: vtl280726p@meta.ua, nvbukalova@gmail.com, nadiyabogatko@ukr.net

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### KEYWORDS

*microbiological criteria,  
safety, food, HACCP,  
technological processes,  
operators of the market  
of food products  
(OMFP).*

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### SUMMARY

Compulsory microbiological criteria should be applied only in relation to those products or in those points of the food chain, which cannot be applied to other, more efficient ones. As well as in the cases, when the microbiological criteria is expected to increase the protection degree offered to the consumer. Microorganisms that make up the components of the criteria must be generally recognized, for example pathogens or indicator organisms that concern the specific food products and technological processes. The obtained results in terms of using the improved method of *Listeria monocytogenes*, *Salmonella* and coagulase-positive *Staphylococcus* detection in milk and dairy products were steady and authentic, so these figures can be used in evaluating the safety of milk and dairy products. In addition, it should be noted that the techniques are simple, economical in execution and its results give certain quality indicators.

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## 1. INTRODUCTION

In 1997 «Kodeks Alimentarius» committee «lished a reference book» «Guidelines for the definition and application of microbiological criteria of foodstuff SAS/GL 21–1997». The Scientific Committee on foodstuff (SCF) and the Scientific Committee on veterinary measures that concern the public health (SCVPH) provided the recommendations on principles of the development of microbiological criteria for foodstuff in 1996 and 1997. According to the definition of «Kodeks Alimentarius» Committee the microbiological criteria for foodstuff determines the acceptability of the food product according to the presence or absence of a number of microorganisms, including parasites or a number of their toxins (metabolites) per unit of weight, volume, area or the production lot. The main principles that are highlighted in these three documents are based on the fact that microbiological criteria will need to be developed and applied only where there is a flagrant necessity and their application is appropriate [1, 3, 5,].

Committee regulations № 2073/2005 determine that to correspond the microbiological criteria the food market operators should develop a program of sample collection and Microbiological studies of these trial [4, 12 16]. The program must be part of the

implementation procedures, developed on the basis of proper hygienic practices and HACCP system principles. The frequency of sample collection should be based on the analysis of risks, should keep in scale with the character and size of the food enterprise, and also should take into account other factors, such as the properties of raw materials, finished product, manufacturing process, etc.

The microorganism may be in raw food, such as fresh meat, raw milk and fish [2, 3, 5].

EU Member States are obliged to apply the microbiological criteria highlighted in Commission Regulations (EC) number 2073/2005. In this document pathogens are directly connected with the type of food product. These pathogens may be present in other types of food products (for example, in bacillus cereus). Besides, there are certain pathogens that are not regulated by EU legislative system (for example, Campylobacter, Clostridium perfringens). In such cases, EU Member States can adopt the national legislation or guidelines that regulate food production at the national level.

There are specific requirements for the microbiological criteria for food safety that can only be used within domestic marketing Ukraine. However, these criteria cannot be used in terms of exporting the food products to the EU market [1], [6].

Therefore, the purpose of our research is the development of bacteriological research methods in milk and dairy products.

## **2. METHODS**

The basis of the horizontal method of *Listeria monocytogenes* determination in milk and dairy products is developing the strategies for improving the horizontal method of *Listeria monocytogenes* determination in milk and dairy products by using the research suspension.

The basis of *Salmonella* detection method in milk and dairy products is developing the strategy for improving the horizontal method for the detection of *Salmonella* in milk and dairy products by changing the quantity of research suspension, which is prepared in the ratio of 1:5 (samples of milk and dairy products in the amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of preview concentration medium).

The method of coagulase-positive staphylococci detection in milk and dairy products is aimed to develop the strategy of improving the horizontal method of coagulase-positive *Staphylococcus* detection in milk and dairy by changing the quantity of used research suspension, which is prepared in the ratio of 1:5 (samples of milk and dairy products in the

amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of previous concentration selective environment [17], [18], [19].

### 3. RESULTS

The common strategy for the implementation of microbiological criteria in accordance with the Regulation of the European Parliament and the Council (EC) № 852/2004 on the hygiene of foodstuffs, which includes the definition of microbiological criteria used in the legislation of the Community; principles of the development and application of criteria and proposals for further measures were developed in EU countries [2, 7, 12, 13]. The mentioned microbiological criteria indicate the acceptability of food products and processes for their production. However, the application of microbiological criteria has certain limitations. Due to reasons connected with sample selection, methodology and uneven distribution of microorganisms, only microbiological studies can never guarantee the safety of the food products that are investigated. Thus, the safety of food products can be guaranteed by application of structured preventive approach that provides the proper product and its production process, as well as the use of proper hygienic practice (GHP) and analysis of dangerous factors and critical control points (on the basis of HACCP system) represented in Regulations № 852/2004 on the General sanitary rules and Regulations № 853/2004 on the approval of special hygiene rules for foodstuffs of animal origin [3, 4].

Microbiological criteria consist of the following components:

- definition of certain microorganisms or their toxins/metabolites and adverse effects that they can cause;
- analytical research methods and standard deviation in case it is reasonable;
- the plan, which specifies the number of sample and the size of the analytic calunit;
- microbiological limits that belong to the food product at certain unit of the food chain;
- the number of analytical units must meet these demands.

Microbiological criteria shall also define:

- food stuff this criterion is applied to;
- the unit of the food chain, the criterion is applied in;
- measures to be taken in case the criterion is not used.

Microbiological criteria can be used differently depending on the place of its application and the procedure to be taken in case of inadequacy.

The criteria set for end products (food safety criteria) can be applied to food products ready for distribution to the market or already distributed to the market that are applied at the stage of food products sale, delivering them both to the end users and retailers. These criteria are applied at points of foodstuff entry at the territory of the EU in the case of its imports from the third countries.

The defined hygiene criteria for the technological processes are applied to food enterprises that manufacture or produce food. They are set for a food product at certain stages of its manufacturing and are not applied to foodstuffs that are already distributed to the market, which are usually used to test the technological processes of production and manufacture of food products, and, for example, may indicate the observance of proper hygienic practices and help to understand the level of HACCP system functioning.

Food safety criteria are mandatory in themselves, and hygiene criteria of technological processes are more like recommendations. Failure in following the mandatory type of criteria leads to disproducts, sorting, recycling or removal of certain food products or their production lots out of the market. Failure in following the criteria in the form of recommendations usually leads to using the correcting activities to the process of food processing. Contents of the correcting activities are determined by market operators of food products.

Microbiological criteria are usually not suitable for monitoring the critical limits defined in HACCP program. Monitoring procedures should be capable of detecting the loss of control in terms of critical points and ensuring the timely provision of such information for the use of correcting activities in order to restore control. Therefore, the measurement of physical and chemical parameters (e.g., acidity, pH, water activity) that can be carried out at enterprises in real time should be used instead of research on the subject of compliance with the microbiological criteria.

Committee Regulations № 2073/2005 define the microbiological criteria for certain pathogens in certain foodstuffs, but also establish criteria for *Listeria monocytogenes* to all ready-to-consume food. So, the food safety criteria are defined in mince and semi-finished raw products (*Salmonella*); gelatin and collagen (*Salmonella*); cheese, butter and sour cream, made from raw milk (*Salmonella*, *Staphylococcus* – enterotoxin); dry milk and dry whey (*Salmonella*); ice cream, made of milk (*Salmonella*); egg products (raw) (*Salmonella*); boiled crayfish and shellfish (*Salmonella*); live bivalve shellfish and living echinoderms (*Salmonella*, *E. coli*); the seedlings seeds (*Salmonella*); dry mixtures for babies and dry dietary foods for special health care needs (for babies under 6 months) (*Salmonella*, *Cromobacter*); dry mixtures for babies over the age of 4 months (*Salmonella*).

Criteria of hygiene processes are determined in mince (number of aerobic colonies) pasteurized milk and pasteurized liquid dairy products (enterobacteria) cheese made from milk or whey that has undergone heat treatment (*E. coli*); cheese made from raw milk or milk treated thermally at temperatures below the temperature of pasteurization (coagulase-positive staphylococci); butter and sour cream (*E. coli*); dry milk and dry whey enterobacteria and coagulase-positive staphylococci); ice cream and frozen dairy desserts (enterobacteria); dry mixtures for babies over the age of 6 months and dry mixtures for babies over the age of 4 months supply (enterobacteria and probable *Bacillus cereus*); egg products (enterobacteria); chopped fruits and vegetables (ready-to-consume) (*E. coli*); not a pasteurized fruit and vegetable juices (ready-to-consume) (*E. coli*).

For the most of the criteria a certain type of food is specified. This does not concern *Listeria monocytogenes* that can be connected with almost all ready-to-serve products. *Listeria monocytogenes* is a pathogen that is well tolerated with food and can cause human diseases. *Listeria monocytogenes* is often met in the environment such as soil, vegetation and animal excrements. The widespread distribution and increased in comparison with other organisms ability to grow or survive in refrigerated environments does *Listeria monocytogenes* a significant risk factor in the production of food products, especially it concerns ready-to-use food that is not treated in the process of production, as well as food that may be contaminated through the environment, including the production environment, in the process of its producing.

That is why it is very important:

- for manufacturers of ready to use food products (designed by their manufacturer for direct human consumption without thermal food processing to destroy or reduce the number of microorganisms) to use different control measures for *Listeria monocytogenes* and its growth in food until the end of the term;
- to accumulate knowledge about the potential bacterial growth in food and to document these facts. The manufacturer must take it into account when determining a safe food expiration date;
- shelf life period is the time during which food product remains safe and meets the requirements of quality in terms of sticking the conditions of its storage and use.

The product date of expiry determines its durability and is expressed by «best before» that is labeled to the food product in accordance with purviews number 9 and 10 of the guideline 2000/13/EC [2,3].

Committee Regulation N 2073/2005 contains criteria for *Listeria monocytogenes* in foodstuffs (table 1).

**Tab. 1. CRITERIA FOR LISTERIA MONOCYTOGENES IN FOODSTUFFS**

Ready-made food products for babies and ready-made meals for special health care needs	
The plan of sampling	$n = 10, c = 0$
Limit	$M = m =$ absence in 25 g
Stage	products placed on the market, for their validity
Ready-made food products, capable to support the growth of <i>Listeria monocytogenes</i> bacteria	
The plan of sampling	$n = 5, c = 0$
2 limits	100 cfu/g or absence in 25 g
2 stages	products distributed to the market, for their validity, or before sending food from the (ORHP)
Ready-made meals not capable to support the growth of <i>Listeria monocytogenes</i> bacteria	
The plan of sampling	$n = 5, c = 0$
Limit	100 cfu/g
Stage	products distributed to the market, for their validity

If necessary, the food market operators, responsible for food production, are obliged to monitor the criteria of safety during the product shelf life. In particular, it concerns ready-made meals that are able to maintain the growth of *Listeria monocytogenes*, which, in its turn, may become peril to the public health. The following guidance [2], [3], [5] were developed to help the representatives of food industry to make researchers on the ability to maintain the growth of the pathogen. These recommendations include:

- the determination of physical and chemical properties of the food product, such as level of acidity (pH), the activity of water ( $a_w$ ), the content of salts, the concentration of the preservatives and the type of packaging, taking into account the conditions of storage and processing, possibilities of pollution;
- to consult on the basis of the existing scientific literature and research on the growth and survival of certain microorganisms.

If necessary, on the basis of the studies, the market foodstuff operators produce additional studies that may include:

- forecasting mathematical modeling for certain food products using the critical factors of the growth and survival of these microorganisms in food;
- research on ability properly seeded certain microorganisms to grow and survive in food in terms of different expected storage conditions;

- research on the growth and survival of certain microorganisms that may be present in food during expire date in terms of prognosticated conditions of distribution, storage and use.

The central referent laboratory for research of *Listeria* has developed guidelines for the investigation of finished food products on their contents [1], [4] for food products, able to support the growth of *Listeria monocytogenes*. The quantity of *Listeria monocytogenes* should not exceed 100 cfu/g by date of the expire.

Committee Regulation (EU) number 2073/2005 indicates the frequency of sample selection of beef, pig, sheep, goat, horse carcasses and poultry with the help of facilities that produce mince and meat semi-finished products. These food products are in danger of being contaminated by microorganismus. Other food products have an average degree of risk.

General recommendations on frequency of sample selection for a group of food products with high and medium-risk listed in table 2.

**Tab. 2.** THE FREQUENCY OF SAMPLE SELECTION OF FOOD PRODUCTS

Risk category of foodstuffs	Large-sized <i>FMO</i> (for international markets, large in size for the internal market in Ukraine)	Medium-sized <i>FMO</i> ( <i>FMO</i> which do not belong to the Group of small and large)	Small-sized <i>FMO</i> and sector services (traditional – small)
High	1 time per week	1 every two weeks	National rules
Average	1 time per month	1 time per 2 months	National rules

Carcasses, fresh meat, meat semi-finished products, ready-made products of certain risk groups (infant formulae) belong to the category of high risk. The rest of the products belong to the average degree of risk include. There is the possibility to reduce sample selection in terms of satisfactory results over a long time (30 weeks or 15 months). Low degree of risk, which includes all other foodstuff, eliminates the requirements of sample selection.

Laboratories that make researchers for food market operators, as well as research methods establishment of microbiological criteria and matrix for the study were accredited (standard of ISO 17025) [3], [4] by National Council for accreditation, or, the equivalent organization, recognized by the European Agency for accreditation or International Association of laboratory accreditation.

The basis of the horizontal method for the *Listeria monocytogenes* detection in milk and dairy is developing the strategies for improving the horizontal method of *Listeria*

monocytogenes detection in milk and dairy products with the help of research suspension, prepared in the ratio of 1:5 (samples of milk and dairy products in the amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of initial selective enriched medium (half of Fraser broth), and further incubation of the suspension for 21–23 hours at temperature of 31±1 °C and secondary enrichment. After the first initial enrichment the received culture in the amount of 0.05–0.06 cm<sup>3</sup> is transferred into the test tube that contains 5–6 cm<sup>3</sup> of second time enriched medium (Fraser broth). Then the environment with crops is incubated for 46–48 hours at the temperature of 37 °C. After that the primary (5–6 cm<sup>3</sup>) and the secondary (2,5–30 cm<sup>3</sup>) enriched culture in terms of selective environment PALKAM-agaris in oculatandis carried out to get clearly separated colonies of *Listeria monocytogenes* for 24±2 hours at the temperature of 37±1 °C in the form of small green grey or olive green colonies, 1.5–2 mm in diameter, sometimes with a black halo, in 48 hours they are in the form of green colonies, 1.5–2 mm in diameter with deeply sunk center and a black halo around.

**Tab. 3.** INDICES OF IMPROVED HORIZONTAL METHOD OF *LISTERIA MONOCYTOGENES* DETECTION IN MILK AND DAIRY PRODUCTS ON THE BASIS OF THE THIRD EXAMPLE

List of the studied samples of milk and dairy products	Detection of <i>Listeria monocytogenes</i> according to the color and size of colonies on the basis example number 3			
	The quantity of samples	The presence of colonies <i>Listeria monocytogenes</i>	The quantity of samples	The absence of <i>Listeria monocytogenes</i> colonies
Raw Milk (fat content of 3.7%), n=10	n=3	After 24± 2 hours a colony of small (1.5–2,0 mm) green grey or olive green color, sometimes with a black halo. In 46 ± 2 hours (1.5–2,0 mm) of green color with deeply sunk centre and a black halo	n=8	Specific colonies of <i>Listeria monocytogenes</i> are not detected
Pasteurized milk (content of fat 2.8%), n=10	n=0		n=10	
Cream, n=6	n=2		n=4	
Pasteurized Cream, n=5	n=0		n=5	
Cheese, n=6	n=2		n=4	
Curd, n=7	n=1		n=6	
Melted cheese, n=8	n=3		n=5	
Butter, n=9	n=2		n=7	
Spread, n=6	n=1		n=5	

The results of our research showed that *Listeria monocytogenes* colonies were found in 24±2 hours. They were of small size about 1.5–2.0 mm in diameter of grey-green or olive-



green color, sometimes with a black halo. In  $46\pm 2$  hours they were of green color with deeply sunk centre and a black halo in the following samples of milk and dairy products (3 samples of raw milk and melted cheese; in 2 samples of cream, melted cheese and butter cream; 1 sample of cheese and spread). Specific colonies of *Listeria monocytogenes* were not founding samples of milk and pasteurized cream. The facts were steady and reliable, therefore, these indicators can be used in evaluating the safety of milk and dairy products. In addition, it should be noted that the method is economic, simple in execution, but its results give specific quality indicators in terms of color and size of *Listeria monocytogenes* colonies.

A method we propose is a qualitative technique of improving the horizontal method of *Listeria monocytogenes* detection in milk and dairy products along with other methods of determining dairy products safety (determination of the total number of microorganisms) and determining the bacteria of intestinal stick group, salmonella and staphylococci) [4], [5].

The advantage of this method among existing qualitative techniques is determining the safety of milk and dairy products on the basis of reliable coloring and size indicators of *Listeria monocytogenes* colonies.

The basis of Salmonella detection method in milk and dairy products is developing a strategy of improving the horizontal method of Salmonella detection in milk and dairy products by changing the quantity of research suspension, which is prepared in the ratio of 1:5 (samples of milk and dairy products in the amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of preview concentration medium (buffered peptone water), and further incubation of received suspension for  $16\pm 2$  hours at the temperature of  $35\pm 1$  °C and subsequent selective concentration. The received culture in the quantity of 0.06 – 0.07 cm<sup>3</sup> is transferred into test tube that contains 5.0–5.1 cm<sup>3</sup> RV medium (chloride of malachite medium of green Rappaport-Vassiliadis) and 5–6 cm<sup>3</sup> of this culture are also put in a flask containing 50–51 cm<sup>3</sup> of selenidecystine medium. These two sawing mediums are kept in the thermostat at the temperature of  $41\pm 1$  °C for about  $23\pm 1$  hours and at the temperature of  $35\pm 1$  °C for about  $23\pm 1$  hours. After that the culture received with the help of two mediums is inoculated with the help of electronic circuit on the surface of Petri cup in the quantity of 2.0–2,5 cm<sup>3</sup>, which contains a solid selective medium (carbolic acid red brilliant green agar-agar). Then we exposure it at the temperature of  $35\pm 1$  °C for  $23\pm 2$  hours to get isolated typical Salmonella colony of red color in terms of changing the medium from pink to red color.

**Tab. 4.** OBSERVATION INDICES OF ADVANCED HORIZONTAL METHOD OF *SALMONELLA* DETECTION IN MILK AND DAIRY PRODUCTS (EXAMPLE № 3)

Samples of milk and dairy products	Detection of <i>Salmonella</i> in terms of color and size of colonies (example № 3)			
	Quantity of samples	The presence of <i>Salmonella</i> colonies	Quantity of samples	The absence of <i>Listeria monocytogenes</i> colonies
Raw Milk (fat content of 3.7%), $n=10$	$n=7$	Isolated typical colonies of red color <i>Salmonella</i> are not detected	$n=3$	Specific isolated typical colonies of red color <i>Salmonella</i> are not detected
Pasteurized milk (content of fat 2.8%), $n=10$	$n=1$		$n=9$	
Cream, $n=6$	$n=4$		$n=2$	
Pasteurized Cream, $n=5$	$n=0$		$n=5$	
Curd, $n=6$	$n=2$		$n=4$	
Cheese, $n=7$	$n=2$		$n=5$	
Melted cheese, $n=8$	$n=3$		$n=5$	
Butter, $n=9$	$n=4$		$n=5$	
Spread, $n=6$	$n=3$		$n=3$	

The results of our research identified the isolated typical colonies of red color *Salmonella* in  $23 \pm 2$  hours at the temperature of  $35 \pm 1$  °C in the following samples of milk and dairy products: 7 samples of raw milk; 4 samples of cream and butter; 3 samples of melted cheese; 2 samples of curd and cheese and 1 sample of pasteurized milk. The peculiar red *Salmonella* colonies were not detected in pasteurized cream.

The obtained results were steady and reliable, so these figures can be used in evaluating the safety of milk and dairy products. In addition, it should be noted that the method is economical, simple in execution. Moreover, its results give specific qualitative indicators in terms of red color isolated typical colonies of *Salmonella*.

We consider this method to be the productive strategy for improving the horizontal method of *Salmonella* detection in milk and dairy products alongside with other methods of safety determination (determination of the total number of microorganisms, determination of coliform bacteria, *Listeria*, *Staphylococcus*) [5], [6].

The advantage of this method is reliable red color indicator of isolated and typical *Salmonella* colonies.

Such strategy of improving the horizontal method of *Salmonella* detection in milk and dairy products differs from other methods by using the research suspension, which is prepared

in the ratio of 1:5 (samples of milk and dairy products in the amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of previous concentration medium (buffered peptone water), and further incubation of received suspension for 16±2 hours at the temperature of 35±1 °C and subsequent selective concentration. The received culture in the quantity of 0.06–0.07 cm<sup>3</sup> is transferred into test tube that contains 5.0–5.1 cm<sup>3</sup> RV medium (chloride of malachite medium of green Rappaport-Vassiliadis) and 5–6 cm<sup>3</sup> of this culture are also put in a flask containing 50–51 cm<sup>3</sup> of selenidecystine medium. These two sawing mediums are kept in the thermostat at the temperature of 41±1 °C for about 23±1 hours and at the temperature of 35±1 °C for about 23±1 hours. After that the culture received two mediums is inoculated with the help of electronic circuit on the surface of Petri cup in quantity of 2.0–2.5 cm<sup>3</sup>, which contains a solid selective medium (carbolic acid red brilliant green agar-agar). And we exposure it at temperature of 35±1 °C for 23±2 hours to get isolated typical Salmonella colony of red color in terms of changing the medium from pink to red color.

The method of coagulase-positive staphylococci detection in milk and dairy products is aimed to develop the strategy of improving the horizontal method of coagulase-positive staphylococci detection in milk and dairy products by changing the quantity of research suspension.

The experiment is done with the help of research suspension, which is prepared in the ratio of 1:5 (samples of milk and dairy products in the amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of selective medium of previous concentration (Giolitti-Cantoni broth and Tween 80), followed by incubation of received suspension for 18±2 hours at the temperature of 35±1 °C. After that the culture of received suspension in the quantity of 1.0–1,1 cm<sup>3</sup> is inoculated on the surface of Petri cup, which contains Beard-Parker agar medium, by rubbing with the help of spatula of inoculum on the surface of agar. Agar is dried in a cup with closed lid for 10–12 minutes at room temperature (20±2 °C). Then the process of incubation takes place: a cup of Petri is put upside down at the thermostat and is left for 24±1 and 48±1 hours at the temperature of 35±1 °C to receive the typical colonies of coagulase-positive staphylococci for 24±1 hours in the form of black or grey, shiny and bulging, 1.0–1.5 mm in diameter (48±1 hours – 1.5–2.5 mm in diameter) and surrounded by clean area and has opalescence rings in 24±1 hours of incubation.

**Tab. 5. INDICES OF ADVANCED HORIZONTAL METHOD OF COAGULASE-POSITIVE STAPHYLOCOCCIDETECTION IN MILK AND DAIRY PRODUCTS (EXAMPLE № 3)**

Studied samples of milk and dairy products	Detection of coagulase-positive staphylococci by color and size of colonies (example № 3)			
	Number of samples	The presence of colonies of coagulase-positive staphylococci	Number of samples	The absence of colonies of coagulase-positive staphylococci
Raw Milk (fat content is 3.7%), <i>n</i> =10	<i>n</i> =2	There are typical colonies of black or grey, shiny and bulging, with a diameter of 1.0–1.5 mm in 24±1 hours (in 48±1 hours – with a diameter of 1.5–2.5 mm) surrounded by a clean area, which in 24 hours of incubation has opalescence rings	<i>n</i> =8	Atypical colonies of shiny black with/without narrow white edge, a pure zone is absent, opalescence ring is absent or barely noticeable; grey colonies without clean areas
Pasteurized milk (fat content is 2.8%), <i>n</i> =10	<i>n</i> =0		<i>n</i> =10	
Cream, <i>n</i> =6	<i>n</i> =3		<i>n</i> =3	
Pasteurized Cream, <i>n</i> =5	<i>n</i> =0		<i>n</i> =5	
Curds, <i>n</i> =6	<i>n</i> =1		<i>n</i> =5	
Cheese, <i>n</i> =7	<i>n</i> =1		<i>n</i> =6	
Melted cheese, <i>n</i> =8	<i>n</i> =1		<i>n</i> =7	
Butter, <i>n</i> =9	<i>n</i> =2		<i>n</i> =8	
Spread, <i>n</i> =6	<i>n</i> =2		<i>n</i> =4	

The results of the research showed that the typical coagulase-positive staphylococci were identified in 24±1 and 48±1 hours at the temperature of 35±1 °C in the following samples of milk and dairy products: 3 samples of cream; 2 samples of raw milk, butter, cream and spread; 1 sample of curd, cheese and melted cheese. The typical colonies of coagulase-positive staphylococci were not found in pasteurized cream and pasteurized milk.

These results were steady and reliable, so these indices can be used in determining the safety of milk and dairy products. In addition, it should be noted that the method is economical, simple in execution, but its results give specific quality indicators of typical colonies of coagulase-positive staphylococci of black or grey color, brilliant and bulging, 1.0–1.5 mm in diameter (in 48±1 hours – 1.5–2.5 mm in diameter) and surrounded by clean area, which has opalescence rings in 24±1 hours of incubation.

We consider this method to be a high-quality strategy for improving the horizontal method of coagulase-positive staphylococci detection in milk and dairy products on an equal basis with other methods of safety determination (determination of the total number of microorganisms, the determination of coliform bacteria, *Listeria*, *Salmonella*) [5].

The advantage of this method is reliable red color indicators of isolated and typical *Salmonella* colonies.

The method of improving the horizontal method of coagulase-positive staphylococci detection in milk and dairy products differs from other methods by using the research suspension, which is prepared in the ratio of 1:5 (samples of milk and dairy products in the amount of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> of selective medium of previous concentration (Giolitti-Cantoni broth and Tween 80), followed by incubation of received suspension for 18±2 hours at the temperature of 35±1 °C. After that the culture of received suspension in the quantity of 1.0–1,1 cm<sup>3</sup> is inoculated on the surface of Petri cup, which contains agar medium of the Beard-Parker. Then it is kept at room temperature (20±2°C) for 10–15 min and incubated at the thermostat at the temperature of 35±1 °C for 24±1 hours and 48±1 hours to get the typical colonies of coagulase-positive staphylococci for 24±1 hours in the form of black or grey, shiny and bulging, 1.0–1.5 mm in diameter (in 48±1 hours – 1.5–2.5 mm in diameter) and surrounded by clean area, which has opalescence ring in 24±1 hours of incubation.

The method, which is based on improving the horizontal method of coagulase-positive staphylococci detection in milk and dairy products, which differs by using the research suspension, which is prepared in the ratio of 1:5 (samples of milk and dairy products in quantities of 10–11 cm<sup>3</sup> (d) and 50–55 cm<sup>3</sup> selective medium of previous concentration (Giolitti-Cantoni broth and Tween 80), followed by incubation of received suspension for 18 ± 2 hours at the temperature of 35 ± 1 °C. After that the culture of received suspension in the quantity of 1.0–1,1 cm<sup>3</sup> is inoculated on the surface of Petri cup, which contains agar medium of the Beard-Parker. Then it is kept at room temperature (20±2°C) for 10–15 min and incubated in the thermostat at the temperature of 35±1 °C for 24±1 hours and 48±1 hours to get the typical colonies of coagulase-positive staphylococci for 24±1 hours in the form of black or grey, shiny and bulging, 1.0–1.5 mm in diameter (in 48±1 hours – 1.5–2.5 mm in diameter) and surrounded by clean area, which has opalescence ring in 24±1 hours of incubation.

#### **4. CONCLUSION**

The obtained results were steady and reliable, so these indices can be used in monitoring the safety of milk and dairy products. In addition, the techniques we applied in our research are simple-to-use, economical and their results give definable quality indicators.

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