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## Methods of microbiological analysis: Monitoring and ensuring food safety

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**Abstract.** Modern methods for detecting indicator microorganisms in food production facilitate the early identification of microbiological risks, thereby preventing the contamination of equipment and food products. These methods also enable the timely implementation of sanitary measures, ensuring the microbiological quality of food products. This study focused on the practical application of microbiological techniques for rapid sanitary and microbiological

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monitoring of raw materials, food products, and contact surfaces of technological equipment. Particular emphasis was placed on evaluating highly efficient and cost-effective approaches to ensuring food safety. During production control, methods that simplify microbiological testing are employed, such as using ready-to-use nutrient media, which significantly reduces the time and resources required for microbiological examinations. The study analysed the primary modern nutrient media used for transporting, cultivating, isolating, identifying, and differentiating microorganisms in food products. Modern microbiological methods have been thoroughly analysed, with an evaluation of the quality of nutrient media and the stability of their properties, which directly impact the validity and reliability of microbiological testing of food products. The study identified and evaluated various test systems and the methodologies according to which they have been validated. The use of Petrifilm test plates – ready-to-use nutrient media designed for the quantitative assessment of different groups of microorganisms – has been analysed. These included quantitative testing for yeasts and moulds, *Escherichia coli*, mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), coliform and Enterobacteriaceae during the production of food products, including semi-finished poultry products. The study also highlighted the current validation certificates for these methods and their compliance with both national and international standards, such as ISO 9001, in the field of development and production. Growing attention to food safety and increased quality requirements for microbiological laboratories promote the adoption of advanced methods for analysing raw materials, food products, and surfaces of technological equipment. These methods significantly accelerate the acquisition of microbiological testing results while optimising resource usage. The study of cost-effective and highly efficient tools and rapid sanitary-microbiological monitoring methods enhances the competitiveness of food products

**Keywords:** safety; pathogen identification; test systems; Petrifilm

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## Introduction

The state regulatory framework addressing food safety requires reform, particularly regarding compliance with safety and quality standards by food producers. In Ukraine, efforts are ongoing within the EU4SaferFood 2 project (State Service of Ukraine on Food Safety and Consumer Protection – Development Strategy of the State Service of Ukraine on Food Safety and Consumer Protection, 2024), which aims to harmonise Ukrainian food safety legislation with that of the EU. The project facilitates the development and implementation of key reforms based on EU law in the domain of food safety, including improving Ukraine's state control system to meet EU standards. One of the anticipated outcomes of this collaboration is the provision of enhanced services in the area of food

safety, alongside the reform of the laboratory infrastructure (NGO Agrarian Union of Ukraine. Agropolitical Report, 2022). This reform is expected to highlight the benefits of monitoring food products produced by enterprises adhering to the European farm-to-fork strategy.

Quality control of food products is a critical aspect of production management, safeguarding businesses from the supply of substandard materials and raw ingredients. The selection of control methods and instruments ensures the desired level of food quality, as the testing process demands the highest levels of precision, reliability, and data accuracy (Varenina *et al.*, 2022). Modern, hightech methods for analysing food quality offer significant advantages over traditional techniques,

reducing the number of research stages, costs associated with the production and validation of culture media, and labour requirements. M. Castro *et al.* (2024) highlight that comprehensive approaches to food safety monitoring, combining theoretical and practical aspects, are essential tools. Moreover, these methods play a crucial role in assessing the quality of finished products and providing timely results for implementing sanitary measures.

The implementation of high-tech, cost-effective tools and rapid methods for routine sanitary and microbiological monitoring is a crucial step in enhancing the quality and safety of food products both in Ukraine and internationally. Traditional methods for the qualitative and quantitative determination of indicator microorganisms suffer from several drawbacks, including being labour-intensive, low-throughput, and time-consuming. These methods are often unsuitable for rapid monitoring, especially in medium-sized and small food processing enterprises that lack accredited bacteriological laboratories. In light of these limitations, V. Chiozzi *et al.* (2022) highlight the need for simpler, more objective, and high-throughput tools and methods for the sanitary and microbiological monitoring of raw materials, semi-finished products, and food products.

Given Ukraine's challenging energy situation and the global trend towards resource reduction, minimising water and electricity consumption has become a pressing issue. Since the early 1990s, global practices have increasingly incorporated methods that simplify microbiological testing of food products, alongside traditional methodologies, particularly in production control. The use of ready-to-use nutrient media reduces the time and resources required for microbiological testing (Hartantyo *et al.*, 2023).

The ability to rapidly obtain results from microbiological analysis allows for the prompt release of food products to the market, providing manufacturers with a competitive

advantage. This paper aimed to investigate the practical application of rapid sanitary and microbiological monitoring of raw materials, food products, and equipment surfaces using modern microbiological methods, and to evaluate highly efficient and cost-effective tools. The objective of this study was to systematise microbiological control methods and assess methods for detecting indicator microorganisms in food production, enabling timely implementation of sanitary measures and ensuring the microbiological quality of food products.

## Literature Review

As highlighted by G. Birgand *et al.* (2022), the history of culture media began in 1861 with the work of French microbiologist L. Pasteur, who used beer wort, wine, grape juice, and meat broth as culture media. The aim was to create a fermentation medium. The primary nitrogen sources were ammonium salts; sugar was used as a carbon source for microbial growth, while ash provided essential micronutrients. Pasteur observed that specific chemical properties and individual nutrients within a medium could either promote or inhibit the growth of particular microorganisms. Given the competitive nature of microorganisms for nutrients, certain cultures can exhibit excessive growth. Pasteur's research led to the modern understanding of preventive measures and proactive monitoring of food products, enabling the implementation, scaling, and utilisation of culture media. As noted by T. Giles-Vernick *et al.* (2022), this distinguished scientist developed microbiological culture media from initial work as a sideline to meat production to the onset of mass production, and to the ongoing development required to meet the demands of modern microbiology laboratories.

As highlighted by H. Craven *et al.* (2021), German bacteriologist R. Koch demonstrated a new technique for using culture media at the London International Medical Congress. At the time, the challenges of using broth-based

culture media were well recognised. Scientists were actively seeking and researching reliable alternatives to replace existing media. Coagulated egg white, starch paste, and aseptically sliced potato were proposed as alternative culture media. In 1881, R. Koch used meat extract with added gelatin to isolate pure cultures of microorganisms. The resulting “nutrient gelatin” was poured into glass plates, inoculated, and placed under a bell jar. This new method of using solid media allowed for both the isolation of pure bacterial cultures and their subculture on plates or gelatin surfaces in tubes sealed with cotton plugs. Although a significant advancement, gelatin had two major drawbacks as a gelling agent. It underwent a gel-to-liquid phase transition at relatively high temperatures, specifically around 25°C, which hindered the incubation of plates at these temperatures. Additionally, gelatin could be liquefied by gelatinase, an enzyme produced by proteolytic microorganisms.

In 1882, Frau Hesse introduced a revolutionary idea: using agar as a replacement for gelatin in the preparation of solid culture media. It is worth noting that this hydrocolloid was already used in the production of fruit jellies at the time. The polysaccharide mixture of agarose and agaropectin possesses unique physical properties: it remains solid up to 85°C but does not solidify into a gel until 34–42°C upon cooling. Agar’s transparency aids in observing colonies, and unlike gelatin, it is not degraded by most microorganisms (Mainardi & Bidoia, 2024).

Meat extract is a valuable source of various growth factors for bacteria, but it has several drawbacks: it does not contain sufficient amino acids to support the optimal growth of many microorganisms; its imprecise chemical composition leads to variable experimental results; and it is susceptible to evaporation. As noted in their book by R. Owusu-Apenten & E. Vieira (2022), F. Loeffler modified the medium by adding peptone and salt to Koch’s original meat extract composition. Peptone was a meat enzymatic digest, used in the 19<sup>th</sup> century as a

pharmaceutical product for digestive disorders. The addition of meat peptone expanded the amino acid composition (as a nitrogen source); salt enhanced the viability of microorganisms, which was crucial for their selective cultivation.

A new type of culture dish for media was introduced in 1887. J. Petri modified a flat glass plate to prevent physical and biological contamination. By the 1890s, nutrient media had been developed that formed the basis for microbiological practice and are still used in modified forms in laboratories today.

Selective media are special nutrient media designed to cultivate specific types of microorganisms. The first selective medium was developed by M. Beijerinck in 1888. Bacteriologist A. McConkey developed the first chromogenic medium in 1905. It contained selective components (neutral red and bile salts) that inhibited the growth of Gram-positive bacteria and a specific substrate – lactose. In their study, R. Singh *et al.* (2020) determined that intestinal bacteria ferment lactose, leading to a decrease in pH and a change in the indicator’s colour. As a result, colonies of *Salmonella*, *Shigella*, and coliform bacteria appear red and are surrounded by a turbid zone of bile salt precipitation. In 1945, A. Fleming, H. Florey, and E. Chain were awarded the Nobel Prize for the development of penicillin, which made it possible to use antibiotics as a practical tool. However, in the 1960s, antibiotics began to be used as selective agents for growing microorganisms, allowing for the avoidance of contamination and creating conditions for the selective growth of specific types of bacteria and fungi.

Chromogenic media contain chromogens, molecules designed to mimic colourless metabolic substrates. These molecules are not cleaved by the target enzyme but accumulate within the cell. Once cleaved, the chromogens become insoluble and coloured, allowing for easy differentiation and identification. The development of selective basal media with chromogenic substrates has facilitated the

creation of advanced nutrient media. As noted by K. Anith *et al.* (2021), this, in turn, has enabled the separation and identification of groups of microorganisms.

In a collaborative study, A. Simpson *et al.* (2022) determined that the use of film-based identification media requires significantly less sample preparation, generates less waste, and occupies less incubator space compared to traditional agar-based methods. Studies have shown that nutrient-coated film plates provide a good correlation for microbial counts with agar-based nutrient media. An additional advantage of film media is the ability to process large sample volumes. This makes them suitable for counting microorganisms in environments with very low biomass, such as extraterrestrial environments. Numerous chromogenic media are now used for various organisms, including *Candida*, *E. coli* and other coliforms, *Listeria monocytogenes*, *Salmonella*, and *Clostridium perfringens*. It is worth noting that much of 19<sup>th</sup>-century microbiology had a clinical focus, but today the food and processing industries face different challenges. There is a need for new approaches in the development and use of specialised methods capable of detecting

minimal quantities of pathogenic microorganisms in 25 g of food products.

## Materials and Methods

The methodology of the study was based on contemporary theoretical and experimental methods of microbiological analysis. The main experimental part of the research was conducted in the laboratory of the Department of Standardization and Certification of Agricultural Products, National University of Life and Environmental Sciences of Ukraine, utilising novel methods and tools for the sanitary and microbiological control of raw materials and food products. Alongside classical culture media for the isolation and differentiation of microorganisms from various taxonomic groups, chromogenic culture media (CCMs) from various manufacturers (Oxoid, Merck, bio Merieux, HiMedia, Sigma, Fluka) were employed. This method is based on the identification of microorganisms based on specific enzymes using special chromogenic substrates.

The study involved the determination of microbiological contamination in two poultry semifinished products. Samples (Fig. 1) included: chicken thigh (broiler), chilled; chicken wing (broiler), chilled.



**Figure 1.** Samples for analysis to determine microbiological indicators

Source: authors' photo

Sample preparation, detection, and enumeration of Enterobacteriaceae in the test samples were conducted according to the ISO 21528 standard on classical culture media. The initial suspension and decimal dilutions

of the test sample were prepared according to ISO 6887. For this, a 10 g sample was aseptically transferred from the combined sample into a sterile filter bag, 90 cm<sup>3</sup> of sterile peptone saline was added, and the mixture was

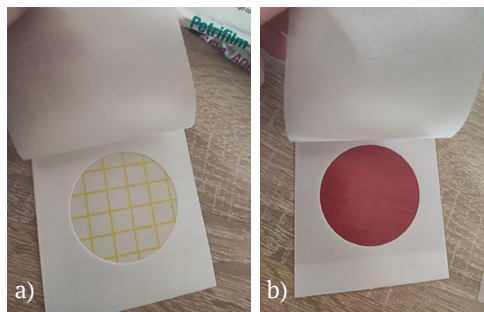
homogenised to obtain the initial suspension. Subsequently, a series of decimal dilutions were prepared from the initial suspension by transferring 1 cm<sup>3</sup> of inoculum into a tube containing 9 cm<sup>3</sup> of sterile peptone saline.

To isolate Enterobacteriaceae, 1 cm<sup>3</sup> of the initial suspension and its decimal dilutions were inoculated into sterile Petri dishes. The inoculum was overlaid with Violet Red Bile Glucose Agar (VRBGA) cooled to 45°C and gently mixed by rotating. After the medium solidified, a second layer of the same medium was poured on top. The plates were incubated at 37°C for 24 hours. Plates containing fewer than 150 characteristic colonies were selected, and these colonies were counted. Five characteristic colonies were then selected for further identification.

The characteristic colonies were transferred to non-selective media and incubated for 24 hours at 37°C. Identification was then carried out by determining oxidase activity and the ability to ferment glucose. Oxidase-negative colonies were inoculated into a glucose agar slant. Inoculated tubes were incubated at 37°C for 24 hours. A positive reaction was indicated by a colour change of the agar to yellow. The number of Enterobacteriaceae per cm<sup>3</sup> or 1 g of the test sample was calculated based on the number of confirmed typical colonies on the plate (in each plate).

Certain species of Enterobacteriaceae may form colourless colonies or cause decolourisation of the medium. Therefore, if no characteristic colonies were observed, whitish colonies were selected for confirmation. The enumeration of Enterobacteriaceae, *Escherichia coli* (SEC), and coliform bacteria was performed using Petrifilm (EC) test plates (Fig. 2).

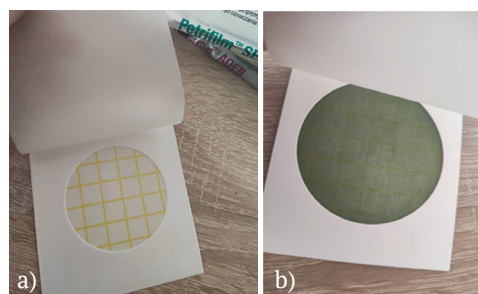
Petrifilm Yeast and Mold Count Plates (Fig. 3) were used to enumerate yeast and mould colonies and to determine lactic acid bacteria. The initial suspension and decimal dilutions of the test sample were prepared according to ISO 6887.



**Figure 2.** Petrifilm test plates for colony counting

**Note:** a – *Escherichia coli* (SEC); b – coliform bacteria and *Escherichia coli*

**Source:** authors' photo



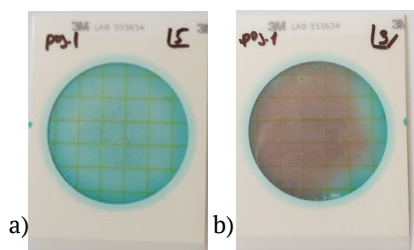
**Figure 3.** Petrifilm test plates

**Note:** a – Yeast and Mold Count Plate for counting yeast and mould colonies; b – determination of lactic acid bacteria

**Source:** authors' photo

The Petrifilm EB plate was placed on a flat surface. The top film was lifted, and 1 cm<sup>3</sup> of the suspension or its dilution was applied to the centre of the bottom film. The film was gently lowered onto the sample, avoiding the entrapment of air. The inoculum was then spread over the surface of the Petrifilm EB using a spreader. The Petrifilm plates were left for one minute to allow the gel to form. Petrifilm EB plates were incubated in a horizontal position with the clear side up at 37°C for 24 hours. The Petrifilm plate for the determination of lactic acid bacteria contains a compound that absorbs oxygen and creates anaerobic conditions. Heterofermentative lactic acid bacteria are identified

as red colonies with gas bubbles. Red colonies without gas are identified as homofermentative lactic acid bacteria (Fig. 4).



**Figure 4.** Petrifilm test plates

**Note:** Petrifilm test plates for the determination of lactic acid bacteria

**Source:** authors' photo

Petrifilm plates for the detection of lactic acid bacteria exhibited an excessive number of colonies, characterised by numerous small colonies, abundant gas bubbles, and a colour change of the gel from blue to pinkish-violet. The high concentration of colonies led to the entire plate becoming dark blue-violet with a pink halo around the outer edge. To obtain a more accurate count, further dilution of the sample was necessary.

Following incubation, results were recorded. In cases where more than 100 colonies were observed on Petrifilm EB plates, the number of colonies in two or more characteristic square areas was counted, and the mean value for each area was determined. To estimate the total number of colonies on each plate, the average number of colonies per square area was multiplied by 20, as the area of the circular inoculation zone is approximately 20 cm<sup>2</sup>. The total number of yeasts and moulds serves as an indicator of the overall cleanliness of food products throughout their lifecycle, from production and processing to distribution and storage. It is important to note that after use, the test systems contain viable cultures of yeasts and/or moulds and should therefore be autoclaved at 120°C for 15 minutes.

## Results and Discussion

The quality of culture media and the stability of their cultural properties directly influence the reliability of microbiological testing of raw materials, semi-finished products, and culinary products. Research conducted by scientists has evaluated the applicability of methods and test systems, particularly Petrifilm indicator plates, for counting microbial groups in food products and on processing surfaces. Researchers M. Bilan *et al.* (2024) conducted a study using traditional microbiological methods and indicator test plates to assess the safety of goat's milk. The results of the study showed that the level of bacterial contamination of goat's milk with mesophilic aerobic and facultatively anaerobic microorganisms was within acceptable limits. No significant differences were found in the results when using alternative methods. The study demonstrated the potential of using test plates to ensure the quality and safety indicators during the production of goat cheese and other dairy products made from it.

The aim of the research by M. Morin *et al.* (2021) was to evaluate the accuracy of the Petrifilm system for detecting excessive bacterial contamination in colostrum. Using Petrifilm for bacterial contamination analysis, the results demonstrated that the test systems were a reliable alternative to standard laboratory methods, with high sensitivity and specificity for detecting aerobic and coliform contamination. The results presented by authors A. Ríos-Castillo *et al.* (2021) indicate that production surfaces and commercial equipment are a source of food contamination. As products can become recontaminated from these surfaces, it is necessary to maintain hygiene standards in retail settings. Given the large number of employees, food products, sales volumes, and customer flow, it is crucial to maintain strict hygiene standards in retail settings to prevent foodborne outbreaks. Procedures must be effective in ensuring food safety.

In collaborative research on frozen food products, M. Silbernagel *et al.* (2003) conducted

comparative studies using the 3M Petrifilm Staph Express Count Plate and the official AOAC 975.55 method for enumerating *Staphylococcus aureus*. Frozen semi-finished products such as lasagna, custard, vegetable mixes, mushrooms, and breaded mushrooms were selected as samples, and the presence of *S. aureus* was determined. The obtained values for contamination of ready-to-eat food products using the 3M Petrifilm Staph Express Count Plate method were similar to the official standard method, guaranteeing the reliability of the results.

L. Nero *et al.* (2020) aimed to assess the applicability of Petrifilm plates for enumerating lactic acid bacteria in bacon. Bacon samples were analysed using four protocols, including conventional control protocols and Petrifilm test plates. Based on the reliable results obtained, researchers determined the ease and speed of using Petrifilm. They verified the adequacy, selectivity, and similarity of results to official protocols, establishing the applicability of alternative indicator plates for microbiological testing of bacon.

M. Sharan *et al.* (2022) emphasised the importance of developing standard procedures for disinfection and hygienic treatment of equipment and utensils to prevent and control microbiological contamination and highlighted the priority of continuous sanitary monitoring. To prevent biofilm formation, it is essential to systematically identify production areas and food processing locations and monitor the microbial load in these zones. They stressed the importance of understanding the mechanisms of microbiological contamination and developing new, cost-effective control methods in the food industry.

Scientists at the US Food Safety Center, S. Jones *et al.* (2020), identified the advantages and disadvantages of bacterial enumeration methods depending on the type of production surface. However, researchers noted the inconsistency of sampling methods in real-world processing plant conditions, which are reliably ensured in laboratory settings. There is a demand

for rapid and reliable methods for assessing bacterial contamination of surfaces to ensure comprehensive food safety and thus guarantee the effectiveness of sanitation procedures.

In their research, M. Gaare & S. Mishra (2021) determined that to prevent food contamination resulting from contact with various production surfaces, a microbiological monitoring program should be developed. The program involves several steps, including a proper procedure for sanitising production equipment, disinfecting production equipment and work surfaces. The next step is to identify potential microbiological hazards during food production or processing, to ensure that production and product distribution comply with international standards. The goal of the research by A. Schumacher *et al.* (2022) was to compare the identification of microorganisms for different 3M Petrifilm test systems. The authors recognised the use of YM as a reliable method for counting viable yeasts and moulds in dried cannabis flowers. They established that 3M Petrifilm YM test systems provide convenience for conducting research and save space in the incubator.

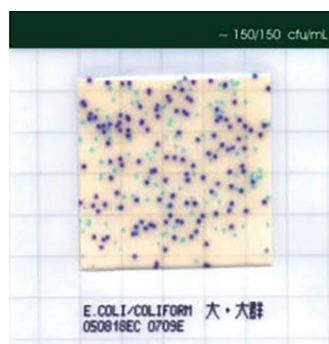
In recent times, test systems in the form of plates (cards) have gained widespread use. The most common among them are Petrifilm, Rida Count, and Compact Dry test systems. Depending on their purpose (detection of specific types of microorganisms), the composition of the nutrient medium is formulated during the production of the test plate. During manufacturing, the nutrient medium is applied to the surface of a mesh polymer base. The nutrient medium contains a special substance that, when a liquid phase (dilutions) of the product is added at room temperature, transforms into a gel. In addition, depending on the type of microorganism, an indicator substance is added to the nutrient medium during the production of the test plates, which colours the colonies a specific colour.

Following the testing methodology, inhibitory substances were added to the nutrient

medium, such as antibiotics (novobiocin), 2,3,5-triphenyl tetrazolium chloride, as well as sodium deoxycholate, bile, and other agents that suppress the growth of accompanying microflora. Chromogenic plates contain substrates that change the colour of the nutrient medium under the action of enzymes produced by specific types of microorganisms. For example, lysine, which is decarboxylated to cadaverine, is added to the medium for the isolation of *Salmonella*. This raises the pH and produces hydrogen sulphide, which, when combined with iron, forms iron sulphide, colouring the colonies black. Chromogenic substrates that are broken down by the enzyme  $\beta$ -galactosidase to form blue colonies are included in the nutrient medium of plates for the isolation of coliform bacteria. COMPACT DRY medium for the detection of *E. coli* contains a substance that, when cleaved by the enzyme glucuronidase present in *E. coli* cells, causes the colonies to turn blue.

**RIDA COUNT.** The monitoring method utilises ready-to-use sterile dry test cards. These systems consist of essential components: nutrient media and chromogenic substrates, specific to particular determinations. The cards are hermetically sealed with an impermeable membrane that is removed before testing. After applying the sample to the card, it is resealed with the same film, ensuring sterility, and then incubated horizontally at the specified temperature. Following incubation, the coloured colonies are counted, taking into account the dilutions and initial volume of the medium. The range of test systems is quite diverse and constantly expanding, including RIDA COUNT TOTAL, RIDA COUNT COLIFORM, RIDA COUNT *E. COLI*, RIDA COUNT *E. COLI* / COLIFORM, and RIDA COUNT *STAPH. AUREUS*. These test systems are designed to determine the quality and assess the microbiological indicators of food raw materials, finished products, animal feed, and swabs from various objects. Currently, test systems are actively used to research the possibility of determining the level of

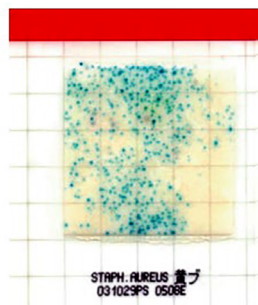
microbiological cleanliness of various production surfaces. The RIDA COUNT plate for the simultaneous detection of coliforms and *E. coli* contains an enzyme that, when interacting with their metabolic products, colours the former blue and the latter purple, as shown in Figure 5.



**Figure 5.** RIDA COUNT plate, *E. coli/coliform*, colony count after 24 hours

**Source:** RIDA COUNT

To detect *S. aureus*, mannitol salt agar is added to the RIDA COUNT plate. This medium contains sodium chloride and antibiotics that inhibit the growth of many microorganisms except staphylococci. The fermentation of mannitol is linked to the acid phosphatase activity of staphylococci and leads to a decrease in pH, which in turn causes the colonies of staphylococci to turn a greenish-blue or black with a green tint, as shown in Figure 6.



**Figure 6.** RIDA COUNT plate, *Staphylococcus aureus*, colony count after 24 hours

**Source:** RIDA COUNT

Petrifilm test plates have certificates of certification from AOAC INTERNATIONAL Official Method of Analysis, AOAC Performance Tested Method<sup>SM</sup>, NF Validation by AFNOR Certification, as well as Ukrainian State Standards DSTU 7089-2009 and DSTU 7140-2009 for the dairy industry. Neogen Petrifilm test plates are

designed for the quantitative determination of Enterobacteriaceae; coliforms; *E. coli*; lactic acid bacteria; yeasts and moulds; mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM); *Staphylococcus aureus*. Table 1 lists the different types of test systems and the methods according to which they are validated.

**Table 1. Validation Certificates for Neogen Petrifilm**

Neogen® Petrifilm®	International standards compliance	DSTU/ ISO Compliance
Neogen Petrifilm for Rapid Coliform Count (RCC)	ISO 4832 <sup>1</sup> , ISO 4832 <sup>2</sup> NF Validation by AFNOR Certification	DSTU ISO 4832:2015 Microbiology of food and animal feed. Horizontal method for coliform enumeration. Colony count technique (ISO 4832:2006, IDT).
Neogen Petrifilm Test Method for Coliform Count (CC)		
Neogen Petrifilm Rapid Yeast and Mould Count (RYM)	ISO 21527 (1-2) NF Validation by AFNOR Certification	ISO 21527-1:2013 Microbiology of food and animal feed. Enumeration of yeasts and moulds. Part 1: Products with water activity greater than 0.95. ISO 21527-2:2008 Microbiology of food and animal feed. Horizontal method for the enumeration of yeasts and moulds. Part 2. Colony count technique in products with water activity less than or equal to 0.95.
Neogen Petrifilm Yeast and Mould Count (YM)		
Neogen Petrifilm for Rapid Aerobic Count (RAC)	ISO 4833 NF Validation by AFNOR Certification	DSTU ISO 4833:2006 Microbiology of food and animal feed. Horizontal method for microbial enumeration. Colony count at 30°C.
Neogen Petrifilm Aerobic Count (AC)		
Neogen Petrifilm Lactic Acid Bacteria Count (LAB)	ISO 15214:2007 NF Validation by AFNOR Certification	DSTU ISO 15214:2007 Microbiology of food and animal feed. Horizontal method for enumeration of mesophilic lactic acid bacteria at 30°C.
Neogen Petrifilm Enterobacteriaceae Count (EB)	ISO 21528-2 NF Validation by AFNOR Certification	DSTU ISO 21528-2:2014 Microbiology of food and animal feed. Horizontal method for detection and enumeration of Enterobacteriaceae. Part 2: Colony count method (ISO 21528-2:2004, IDT).
Neogen Petrifilm Staph Express Count (STX) + Staph Express Disk for Coagulase Activity Confirmation	ISO 6888-1NF Validation by AFNOR Certification	DSTU ISO 6888-1:2003 Microbiology of food and animal feed. Horizontal method for coagulase-positive <i>Staphylococci</i> ( <i>Staphylococcus aureus</i> and others). Part 1: Using Baird-Parker agar medium (ISO 6888-1:1999, IDT).
Neogen Petrifilm <i>E. coli</i> Count (SEC)	ISO 16140-2 NF Validation by AFNOR Certification	ISO 16649-2 Horizontal method for the enumeration of $\beta$ -glucuronidase-positive <i>Escherichia coli</i> .

**Note:** standard names are presented as specified by the responsible Technical Committees

**Source:** developed by the authors

The use of such systems provides a significant (1.5-2 times) reduction in direct costs for conducting analyses and allows for a shorter study duration. Petrifilm plates are designed for the quantitative determination of various groups of microorganisms. They consist of a

nutrient medium on a substrate. Petrifilm has a multilayer structure and comprises a substrate with a nutrient medium; and a transparent film covering the plate to maintain sterility. The media contain necessary components: chromogenic substrates and indicators, which

give the microbial colonies a characteristic colour. Modified substrates are also added to the chromogenic nutrient medium of rapid tests, allowing for the detection of specific biochemical properties of microorganisms. The use of Petrifilm plates begins directly with sample preparation for microbiological analysis. Stages such as preparing glassware and nutrient media, and conducting quality control of the media in independent laboratories for activity and selectivity are eliminated.

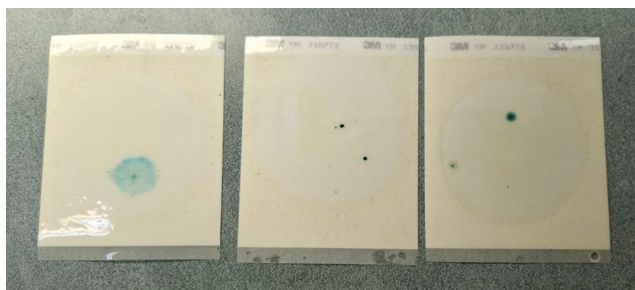
Petrifilm test plates are available in a variety of types, each designed for specific applications. Unopened packages of Petrifilm should be stored in a refrigerator or freezer at a temperature not exceeding 8 °C (46 °F). Before opening a package of Petrifilm, allow it to reach room temperature (20-25 °C, RH < 60%). To avoid moisture exposure, resealed packages should be stored in a cool, dry place for no more than one month. If the laboratory temperature exceeds 25 °C (77 °F) with a relative humidity above 50% (except for air-conditioned rooms), resealed packages of Petrifilm should be stored in a freezer.

Opened packages of Petrifilm should be stored in a tightly sealed container in the freezer. It is not recommended to use Petrifilm plates after the expiration date. Specific requirements for laboratory freezing equipment are recommended. The freezer used to store opened packages should operate without an automatic defrost

cycle to avoid damaging the plates through repeated contact with moisture. Petrifilm plates that have changed colour should not be used.

Petrifilm RAC is a ready-to-use nutrient medium designed for the rapid enumeration of aerobic bacteria. It contains nutrients, a cold-water-soluble gelling agent, and a dual-perception indicator to facilitate colony counting. Petrifilm RAC plates are intended for the enumeration of aerobic bacteria in raw materials, finished products, environmental samples (air, swabs, imprints), and beverages. Petrifilm RAC plates are registered by Neogen for use solely in the food and beverage industry. For example, Petrifilm RAC plates are not registered by Neogen for the analysis of water, pharmaceuticals, or cosmetics. Petrifilm RAC plates are not intended for the diagnosis of human or animal diseases.

Petrifilm RAC plates cannot differentiate between various strains of microorganisms. Petrifilm AC is an Aerobic Count Plate used for enumerating aerobic bacteria in food and beverages. Petrifilm YM is a Yeast and Mold Count Plate designed for quantifying yeasts and moulds. Petrifilm YM is a ready-to-use culture medium containing nutrients, antibiotics, a cold-water-soluble gelling agent, and an indicator to simplify the counting of yeasts and moulds. Petrifilm YM plates are designed for the enumeration of yeasts and moulds in food and beverage production (Fig. 7).

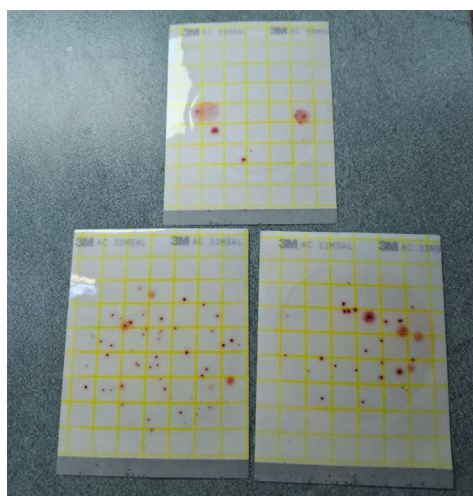


**Figure 7.** Typical colonies of moulds and yeasts

**Note:** typical mould colonies are large with a dark centre and diffuse edges, flat, blue-green or with colour variations; typical yeast colonies are small with smooth edges, convex, pinkishbrown, or blue-green

**Source:** authors' photo

Petrifilm Aerobic Count Plates (AC) are designed to enumerate mesophilic aerobic and facultatively anaerobic microorganisms (MAF-AnM) in food production and on contact surfaces (Fig. 8). They can also be used for environmental monitoring. Using Petrifilm AC reduces labour costs and allows for more frequent monitoring at critical control points in production, ensuring effective food safety and quality control.



**Figure 8.** Petrifilm Aerobic Count Plate (AC) for counting MAFAnM

**Note:** top – 5 CFU, left – 50 CFU, right – 28 CFU

**Source:** authors' photo

Petrifilm *E. coli* Count Plates (SEC) are ready-to-use nutrient media containing selective agents, nutrients, a cold-water-soluble gelling agent, and a glucuronidase indicator BCIG (5bromo4-chloro-3-indolyl-D-glucuronide) to facilitate colony counting. Petrifilm SEC plates are designed for the enumeration of *Escherichia coli* (*E. coli*) in food and beverage production. Most strains of *E. coli* are thermotolerant and produce beta-glucuronidase, an enzyme that reacts with the BCIG indicator in 3M Petrifilm SEC plates, forming a dark green or blue-green substance that colours the colonies.

Each batch of Petrifilm test plates is accompanied by a quality certificate from the

independent laboratory AFNOR. Petrifilm test plates are validated according to standard methodologies. The results obtained from cultures on Petrifilm plates are consistent, reliable, and 100% reproducible. When conducting microbiological tests using Petrifilm plates, compared to traditional methods, there is a 76% reduction in electricity consumption and a 79% reduction in water usage. Studies conducted by the manufacturer show a significant reduction of up to 75% in greenhouse gas emissions and up to 66% in waste. Additionally, the use of Petrifilm plates allows for a 45% reduction in average labour costs and an 80% increase in the efficiency of technical personnel. The accuracy, reliability, and speed of determining food quality allow for increased product competitiveness and rapid release for sale.

Switching from agar to Petrifilm count plates reduces labour costs by an average of 45%. A study of 292 food processing plants showed an average increase in the efficiency of quality control personnel of 80.5% using Petrifilm – or 3.7 hours of saved time for technical personnel per day. By obtaining results within 24 hours or less, Petrifilm can provide data approximately twice as fast as traditional agar plate methods. Stable in storage and as thin as paper, Petrifilm test plates take up 85% less space when incubated. Petrifilm plates use less glassware and equipment and optimise the workspace and incubator space compared to traditional methods. Studies have shown that when conducting quality analysis of raw materials, individual production stages, and the disposal of packaging materials, the use of Petrifilm plates is an energy-saving, labour-saving, and environmentally friendly method.

## Conclusions

Food testing and quality control demand highly sophisticated technology. This article analyses contemporary methods for the microbiological examination of food products; it explores highly efficient and cost-effective tools and

methods for the rapid sanitary-microbiological monitoring of raw materials, food products, and the surfaces of processing equipment. It presents current validation certificates, demonstrating their compliance with the International Organisation for Standardisation (ISO) 9001 standards in research and development. The application of modern validated methods for microbiological monitoring and ensuring food safety optimises turnaround times, enhances the accuracy and reliability of results and reduces energy and labour costs for such studies.

Research has demonstrated that using Petrifilm test plates for microbiological analysis significantly reduces the consumption of electricity, water, and greenhouse gases compared to traditional methods. This leads to a 45% reduction in labour time and an 80.5% increase in staff efficiency. Due to the rapid result delivery within 24 hours, Petrifilm is twice as fast as traditional methods. Test plates also save incubator space and optimise the use of laboratory equipment. Overall, Petrifilm is an energy-efficient, labour-saving, and environmentally friendly method for food quality analysis. The advantages of Petrifilm test plates allow for savings in the time spent preparing media, simplify the testing process through quality identification, and reduce disposal costs. The use of simple, technological tools and objective methods

for the sanitary-microbiological control of raw materials, food products, and the surfaces of processing equipment guarantees food safety.

Future research plans include refining processes for the detection and control of microbiological hazards in the food industry using advanced analytical methods. Continued research is planned to evaluate and select highly effective yet cost-effective sanitary control tools, such as Petrifilm test plates. In particular, further investigation into specific culture media for the quantitative analysis of microorganisms and their compliance with ISO standards will enhance the competitiveness of food products by ensuring their microbiological safety.

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### Conflict of Interest

None.

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## **Методи мікробіологічного аналізу: контроль та гарантування безпеки харчових продуктів**

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**Анотація.** Сучасні методи виявлення індикаторних мікроорганізмів на виробництві харчових продуктів дозволяють завчасно виявити мікробіологічні ризики і попередити контамінацію обладнання та харчових продуктів мікроорганізмами та своєчасно організувати санітарні заходи, тим самим забезпечуючи якість харчової продукції за мікробіологічними показниками. Стаття була спрямована на дослідження практичного застосування мікробіологічних методів для проведення оперативного санітарно-мікробіологічного моніторингу сировинних матеріалів, харчових продуктів та контактних поверхонь технологічного обладнання, з акцентом на оцінку високоєфективних та економічно рентабельних засобів для забезпечення безпечності харчових продуктів. Під час виробничого контролю застосовують методи, що спрощують мікробіологічні випробування харчових продуктів, зокрема використання готових поживних середовищ дозволяє скоротити витрати часу і ресурсів на проведення мікробіологічних випробувань. Проведено аналіз основних сучасних поживних середовищ, що використовуються для транспортування, культивування, виділення, ідентифікації та диференціації мікроорганізмів харчових продуктів. Детально проаналізовано сучасні мікробіологічні методи; здійснено оцінку якості поживних (живильних) середовищ і стабільність їхніх властивостей, що позначається на обґрунтованості й адекватності мікробіологічних випробувань харчових продуктів. У статті визначено та оцінено різновиди тест-систем та методи, згідно з якими вони валідовані. Проведено аналіз застосування тест-пластин Petrifilm – готових поживних середовищ, які призначені для кількісного визначення різних груп мікроорганізмів, зокрема для кількісного аналізу дріжджів та цвілевих грибів, *Esherichia coli*, мезофільних аеробних і

факультативно-анаеробних мікроорганізмів (МАФАНМ), ентеробактерій та колиформних бактерій, під час виробництва харчових продуктів (напівфабрикатів з сільськогосподарської птиці). Наведено сучасні сертифікати їх валідації, їх відповідність в державній та міжнародній організації зі стандартизації (ISO) 9001 у сфері розробок та виробництва. Підвищення уваги до безпеки харчових продуктів та вимог до якості мікробіологічних лабораторій сприяє застосуванню сучасних методів аналізу сировини, харчових продуктів і поверхонь технологічного устаткування. Використання методів дозволяє значно пришвидшити процес отримання результатів мікробіологічних досліджень, а також оптимізувати використання ресурсів. Вивчення засобів, що вирізняються високою ефективністю і невисокою ціною, та методів оперативного санітарно-мікробіологічного моніторингу забезпечить конкурентоздатність харчової продукції

**Ключові слова:** безпечність; ідентифікація збудників інфекцій; тест-системи; петрифільми