

School of Applied Science

Diploma in Medical Biotechnology

**Assessing the Suitability of Petrifilm *Enterobacteriaceae* Count Plates as an Alternative Method to Replace Agar Dishes Preparation in Laboratories**

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1. *Introduction*

1.1 Background information

Foodborne infections remain a significant public health problem worldwide. Foodborne diseases affect an estimated 600 million people annually and can cause hospitalizations, serious health issues, and even death. The main reason for these infections is eating contaminated food, which frequently contains dangerous microbes of which the most common is bacteria.

The Gram-negative bacterium family *Enterobacteriaceae* stands out among the other bacterial families that cause foodborne infections as a significant source of the issue. Numerous pathogenic species, including *Escherichia coli, Salmonella, Shigella,* and *Yersinia* are frequently linked to gastrointestinal infections, resulting in symptoms like diarrhea, cramping in the abdomen, and fever. In addition, the *Enterobacteriaceae* family can result in bacterial meningitis, bloodstream infections, lung infections, wound infections, and urinary tract infections.

The precise and prompt identification of *Enterobacteriaceae* contamination is essential for efficient food safety management since it can happen in several phases of food production, processing, distribution, and preparation. It is crucial to create quick, accurate, and effective microbiological testing techniques to tackle the problems caused by foodborne infections and the presence of *Enterobacteriaceae* in food products.

Petrifilm, a novel alternative, has been developed as a solution to address these limitations by offering ready-to-use, pre-made film strips with particular culture media, which removes the need for labor-intensive hand agar plate preparation, improving standardisation, and saving significant amounts of time and money in labs.

Although there are benefits to utilizing Petrifilm, its complete potential for *Enterobacteriaceae* detection and enumeration in laboratories has not been fully established; therefore, a thorough investigation is necessary to determine its suitability for *Enterobacteriaceae* detection and enumeration

1. 2 Aim

The project's goal is to successfully replace conventional agar plates for Petrifilm in laboratory *Enterobacteriaceae* tests for detection and enumeration. The goal of the study is to increase laboratory efficiency by demonstrating the dependability, precision, and usefulness of Petrifilm as a substitute.

1. *Literature Review*

2.1 Traditional methods used for *Enterobacteriaceae* detection and enumeration

For the purpose of monitoring the safety and quality of food, particularly in food samples, Enterobacteriaceae must be detected and enumerated. Over the years, a variety of techniques have been used, each with advantages and disadvantages of its own. Among the conventional techniques, the normal plate count method, the most probable number (MPN) count, and the direct microscopic count stand out.

The Most Probable Number (MPN) method provides an estimated range of viable microorganisms in a given sample using statistical probability. It's not an exact figure but a likely range. The technique involves adding the sample to different tubes or wells containing growth medium, and after incubation, positive growth tubes help estimate the most likely bacterial count through MPN tables (Basak & Shetty, 2021).

Direct microscopic counting involves observing bacterial cells in a sample under a microscope. A designated volume of the sample is analyzed under the microscope, and the visible bacterial cells help determine the sample's bacterial concentration (Basak & Shetty, 2021).

Through biochemical assays, bacterial species are differentiated based on their metabolic reactions. For Enterobacteriaceae, tests such as indole production, citrate utilization, urease activity, and motility are standard. For example, Escherichia coli shows a positive indole test, setting it apart from many other Enterobacteriaceae (Vasavada et al., 2020).

The International Organization for Standardization (ISO) formulates globally accepted methods for consistent microbial detection across labs and nations. EN ISO 21528 targets Enterobacteriaceae detection and involves steps like initial enrichment in buffered peptone water, cultivation on selective media, and additional tests for confirmation (Biesta-Peters et al., 2019).

Traditional detection methods for Enterobacteriaceae have limitations. They often need long incubation times, which can hinder timely decisions in sectors like food safety or clinical diagnostics. For instance, using VRBG agar might necessitate up to a full day's incubation (Vasavada et al., 2020). In scenarios where rapid response is vital, such as food safety checks, this delay can be problematic.

Routine culture-based tests require meticulous handling and are labor-heavy (Basak & Shetty, 2021). Skilled personnel are essential, adding to costs and time. Any missteps can result in inaccurate outcomes. For instance, the MPN method demands expertise and attention to detail, adding to its complexity and time consumption.

Traditional methods might not detect Enterobacteriaceae in specific samples as effectively as newer methods (Biesta-Peters et al., 2019). Direct microscopic count lacks the precision needed to spot low bacterial counts and to differentiate between Enterobacteriaceae species (Vasavada et al., 2020).

Given their manual nature, traditional methods aren't ideal for analyzing vast samples, making them less suited for extensive research or quick screenings (Basak & Shetty, 2021). Also, methods like the plate count are challenging to scale up. Following EN ISO 21528 protocols can further compound these challenges (Biesta-Peters et al., 2019).

These traditional methods are best suited for detecting well-documented Enterobacteriaceae strains, which may mean they miss new or uncommon strains (Basak & Shetty, 2021).

The design of traditional methods can occasionally produce incorrect results. Conditions might not always be right for specific bacterial growth, leading to false negatives. On the other hand, contamination might result in false positives (Biesta-Peters et al., 2019).

According to the three papers mentioned, the predominant method for identifying and counting Enterobacteriaceae is the standard plate count using VRBG agar. The upcoming study will evaluate the potential of Petrifilm Enterobacteriaceae Count Plates as an alternative to VRBG agar for food sample testing.

2.2 Introduction to Petrifilm

Petrifilm is a novel alternative to traditional methods used in the microbiological testing of food and drinks that has been created by the company 3M. It offers a ready-to-use solution for the quick enumeration of microorganisms (Abgrall & Cleret, 1990). Petrifilm aerobic count plate, Petrifilm Enterobacteriaceae count plate (including Salmonella, Shigella, and Yersinia), Petrifilm coliform count plate, Petrifilm Escherichia coli/coliform count plate, Petrifilm yeast and mold count plate, Petrifilm rapid coliform count plate, and Petrifilm environmental Listeria count plate are all examples of culture films.

Petri dishes, preparing culture media, and other labor-intensive tasks involved in conventional techniques are all done away with by using Petrifilm plates, which also increases the capacity for sample analysis within a set amount of time. Additionally, the use of indicators accelerates colony identification, improving the procedure's accuracy and effectiveness. (Medina & Jordano, 2014)

The dry film Petrifilm plates are ready to use right away; they are made of two plastic films that are securely fastened together along one edge and coated on opposite surfaces with culture media components, such as selective or non-selective agents designed to target particular microbes (Silbernagel and Lindberg, 2003). The set is completed with an indicator dye and a gelling agent that is soluble in cold water. The target region of the plate, which is intended for colony counting, has squares of various sizes that are tailored for use with various products and make enumerations simple. (Medina & Jordano, 2014)

Focusing in mind the 3M Petrifilm Enterobacteriaceae Count Plate that is being studied for this paper, this plate includes adjusted Violet Red Bile Glucose (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that makes colony enumeration easier. It is specially made for counting Enterobacteriaceae, a group of oxidase-negative, Gram-negative rods recognized for their capacity to ferment glucose and produce byproducts such as acid and/or gas. Enterobacteriaceae colonies appear as red with yellow zones, red with gas bubbles, or red with both yellow zones and gas bubbles on the Petrifilm EB Plates, allowing for swift and precise diagnosis. (3M Enterobacteriaceae count plates, n.d.)

In conclusion, the Petrifilm system's broad selection of plates, which includes Enterobacteriaceae, coliforms, E. coli, total aerobic bacteria, yeasts and molds offers complete solutions that do not require antibiotics or difficult enrichment steps (Medina & Jordano, 2014), thus streamlining and speeding up the entire testing process

2.3 Potential benefits and drawbacks of Petrifilm

One of the noteworthy characteristics of Petrifilm plates is their constitution with selective nutrients. These nutrients stimulate the growth of intended microorganisms while hindering the proliferation of non-target ones, thereby enhancing the specificity of the test and reducing the possibility of ambiguous outcomes. (Medina & Jordano, 2014)

An important feature of Petrifilm plates is the incorporation of chromogenic substrates and redox indicators. These constituents are specifically designed to confer distinct colors to microbial colonies, thereby facilitating visual interpretation and analysis of results.

In terms of rapidity, Petrifilm plates are recognized for their swift capability in microbial analysis. They provide prompt results in the quantification and identification of microorganisms, a particularly crucial aspect in sectors where timely decision-making directly impacts the quality and safety of products. (Jasson et al., 2010)

However, it is essential to recognize certain limitations. While Petrifilm plates offer efficiencies in setup and can accommodate a larger number of samples simultaneously, they generally do not reduce the time required to complete each individual assay. This indicates that the overall time-to-result may remain comparable to that of traditional agar plate methods.

Specific challenges may arise when utilizing Petrifilm yeast and mold plates. Accurately distinguishing between yeast and mold colonies can prove to be quite demanding, thus potentially affecting the reliability of results for certain types of samples. (Medina & Jordano, 2014)

Moreover, the presence of organisms that produce mannanase, such as Bacillus amyloliquefaciens, can lead to the liquefaction of the plate. This can subsequently result in inaccuracies in quantification, either through overestimating or underestimating the microbial load present in the sample. (Feng et al., 2017)

Lastly, Petrifilm plates are tailored for specific target organisms, which can present a limitation for broader microbial screenings. In cases where a comprehensive microbial analysis is required, additional testing methods may still be necessary, potentially offsetting some of the efficiency gains offered by the use of Petrifilm. (Medina & Jordano, 2014)

2.4 Studies evaluating Enterobacteriacea Petrifilm Count Plates

In a collaborative study entitled "The 3M™ Petrifilm™ Enterobacteriaceae Count Plate Method for Enumeration of Enterobacteriaceae in Selected Foods," the primary aim was to evaluate the precision and reproducibility of the Petrifilm Enterobacteriaceae Count Plate method. This technique was compared to two established methodologies: the techniques described in the Standard Methods for the Examination of Dairy Products (SMEDP) and the Compendium of Methods for the Microbiological Examination of Foods (Compendium). The study sought to determine whether the Petrifilm Enterobacteriaceae Count Plate method could provide a viable alternative for quantifying Enterobacteriaceae in selected foods.

In this investigation, different food samples underwent analysis using both the Petrifilm Enterobacteriaceae Count Plate method and either the SMEDP or Compendium methods. The findings illustrated that the Petrifilm method produced results that were comparable in accuracy to the Violet Red Bile Glucose Agar (VRBG) method for quantifying Enterobacteriaceae in selected foods, specifically at the moderate level of inoculation. Importantly, at this specific level, the average log10 counts obtained from the Petrifilm plates were not significantly different from those acquired through the VRBG method.

Nevertheless, discrepancies were observed at other levels of inoculation, where the Petrifilm plates exhibited significantly higher counts compared to the VRBG method. Additionally, the repeatability variance of the Petrifilm plate method proved to be significantly superior to that of the VRBG method, particularly at the moderate and high levels of inoculation. This aspect underscored the precision and reproducibility of the Petrifilm method in these particular circumstances.

Overall, the collaborative study concluded that the Petrifilm Enterobacteriaceae Count Plate method offered accuracy and specificity in quantifying Enterobacteriaceae, and it performed similarly to the VRBG method in terms of accuracy and repeatability, particularly at the moderate inoculation level. (Silbernagel and Lindberg, 2003)

In their research article titled "Assessment of the Application of Petrifilm Count Plates for the Quantification of Enterobacteriaceae in Poultry Samples," the authors aimed to evaluate the effectiveness and dependability of the Petrifilm technique in quantifying Enterobacteriaceae in poultry samples. This study presented a unique perspective by comparing the Petrifilm method with traditional quantification techniques that involved sequential decimal dilutions.

The study encompassed several crucial aspects, including examinations of the linearity of the alternative Petrifilm method, statistical analyses such as Chi-square tests, dispersion index (G) values, and correlation coefficients (R^2). The primary objective was to assess the proportional relationship between the results obtained from the Petrifilm method and the concentration of Enterobacteriaceae in the samples.

The results consistently demonstrated the superiority of the Petrifilm system over the traditional method. Notably, the Petrifilm method displayed enhanced consistency in the data obtained compared to the traditional approach, which was essential for achieving accurate quantification. Furthermore, the Petrifilm method produced faster results, eliminating the need for confirmatory biochemical tests, a step typically required in the traditional procedure.

Moreover, the linearity test conducted on the Petrifilm method revealed its capability to generate results that were directly proportional to the concentration of Enterobacteriaceae in the samples, covering a range from 1 to 256 colonies. This proportionality was supported by a high correlation coefficient (R^2), indicating the absence of bias in the method.

In contrast, the traditional method exhibited less robust linearity and proportionality. Specifically, it showed minimal proportionality between dilutions, with the lowest dilution obtained falling below the average used for the linearity test in the alternative Petrifilm method. As a result, the traditional method sometimes failed to accurately quantify Enterobacteriaceae populations in the samples.

Furthermore, the Petrifilm method consistently demonstrated superior consistency and precision compared to the traditional method when quantifying Enterobacteriaceae populations across various dilutions. These findings emphasized the advantages of the Petrifilm method in terms of efficiency, reliability, proportionality, linearity, and consistency, making it a valuable tool in microbiological analysis.

In conclusion, the Petrifilm quantification system excelled in terms of efficiency, reliability, proportionality, linearity, and consistency when compared to the traditional method. It offered speed, eliminated the need for confirmatory tests, and provided improved precision in quantifying Enterobacteriaceae populations. Overall, the study concluded that the Petrifilm Enterobacteriaceae Count Plate method is a viable alternative for quantifying Enterobacteriaceae in poultry samples, offering consistent and reliable results, speed, and the absence of confirmatory tests. Its demonstrated linearity and selectivity make it a comparable choice to traditional methods and suitable for microbiological analysis. (Drebes et al., 2012)

The primary aim of the study titled 'Evaluation of the 3M Petrifilm Enterobacteriaceae Count Plate Method for the Enumeration of Enterobacteriaceae in Foods' was to evaluate the efficacy of the 3M Petrifilm Enterobacteriaceae Count Plate method in quantifying Enterobacteriaceae in food items. This technique was compared to the standard VRBG method, with a particular focus on evaluating the sensitivity and selectivity of the Petrifilm plate method in relation to the VRBG method, specifically in terms of recovering Enterobacteriaceae strains from pure cultures.

The study was structured into four distinct phases: the impact of non-Enterobacteriaceae organisms in pure cultures, recovery of Enterobacteriaceae from pure cultures, detection of Enterobacteriaceae in uninoculated food products, and identification of Enterobacteriaceae in inoculated food products.

In the first phase, Petrifilm plates and VRBG agar were used to culture diluted pure cultures of non-Enterobacteriaceae organisms, followed by incubation. The second phase involved diluting and plating pure cultures of Enterobacteriaceae on Petrifilm plates and VRBG agar, followed by incubation. The third phase consisted of preparing uninoculated food samples from nine different food categories suspected to have high bacterial loads, which were then examined for natural contamination. The fourth phase encompassed artificially inoculated food samples, where both dairy and nondairy food items were contaminated with a specific blend of bacteria at varying levels of inoculation.

The results of this study demonstrated that the Petrifilm plate method exhibited a sensitivity comparable to that of the VRBG method. Specifically, it successfully recovered 60 out of 62 pure Enterobacteriaceae cultures, mirroring the performance of the VRBG method. The only exception was the failure to recover two strains of Yersinia pseudotuberculosis using either method.

Moreover, the Petrifilm plate method demonstrated comparable or superior performance to the VRBG method in quantifying Enterobacteriaceae in both naturally contaminated and artificially inoculated dairy and nondairy food samples. Notably, the Petrifilm plate method yielded higher log counts than the VRBG method for certain food categories, including spices, fresh fish and seafood, ground meats, and poultry rinse. This suggests that the Petrifilm method was able to recover a greater number of organisms in these specific food categories.

The study also examined the proportion of confirmed Enterobacteriaceae organisms between the Petrifilm plate and VRBG plate methods. In most instances, there were no significant differences in proportions. However, ground meat and fish and seafood samples showed a notable distinction, with the Petrifilm plate method detecting more Aeromonas-like organisms.

When considering the mean log counts of Enterobacteriaceae per gram, the Petrifilm Enterobacteriaceae Count Plate method and VRBG method produced similar results for six out of thirteen inoculated food samples.

In conclusion, this study determined that the Petrifilm plate method is a dependable alternative to the VRBG method for quantifying Enterobacteriaceae in food samples. It offers comparable sensitivity, consistent performance, and even surpasses the VRBG method in certain food categories. These findings underscore the suitability of the Petrifilm Enterobacteriaceae Count Plate method for enumeration and detection purposes (Silbernagel & Lindberg, 2002).

1. *Materials and Methods*

**Materials:**

VRBG Agar (Violet Red Bile Glucose Agar)

Petrifilm Enterobacteriaceae Count Plates

Known Enterobacteriaceae strains for spiking

Phosphate buffer saline (PBS)

Serial dilution tubes

Sterile pipettes and tips

Sterile swabs

Sample Matrices:

Raw meat

Cooked food

Powder

Liquid

Swabs

Preserved Meat

Milk Powder

**Methods:**

* Sample Preparation:
1. A total of 10-20 samples from diverse matrices will be gathered.
2. Each sample will be classified into three categories: High count, Low count, and Control.
3. The Control samples will remain unadulterated and will function as a fundamental reference point for the experiment.
* Sample Spiking:

Known quantities of Enterobacteriaceae strains will be introduced into the samples to generate high and low count samples.

* Sample Plating:
1. Utilizing aseptic pipettes, a portion of each spiked sample will be placed onto VRBG agar plates.
2. Another portion of the same sample will be placed onto Petrifilm Enterobacteriaceae Count Plates.
3. All plates will be subjected to incubation at a temperature of 37°C for a duration of 24 hours.
* Sensitivity Testing (using Petrifilm):
1. Serial dilutions of the known Enterobacteriaceae strain will be prepared employing PBS.
2. These dilutions will then be placed onto Petrifilm plates.
3. Subsequently, the plates will be subjected to incubation at a temperature of 37°C for a duration of 24 hours.
4. Following incubation, the colonies on the Petrifilm plates will be enumerated to evaluate the sensitivity of the Petrifilm method.
* Specificity Testing:
1. Non-Enterobacteriaceae strains will be used to spike samples.
2. These samples will subsequently be plated onto both VRBG agar and Petrifilm plates.
3. The growth of non-target organisms will be monitored to ascertain the specificity of each method.
* Data Analysis:
1. The colony counts obtained from both VRBG agar and Petrifilm plates will be compared to evaluate the accuracy and reliability of each method.
2. The results pertaining to sensitivity and specificity will be utilized to determine the efficacy of Petrifilm plates in relation to the conventional VRBG agar method.

4. *Time plan*

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References:

Food safety. (n.d.). Who.int. Retrieved September 22, 2023, from <https://www.who.int/news-room/fact-sheets/detail/food-safety>

(N.d.). Www.3m.com. Retrieved September 22, 2023, from <http://www.3m.com/3M/en_US/food-safety-us/>

Gray, J. M., Jaykus, L. A., & Wang, T. C. (2019). Challenges and Advances in Food Safety Microbiology. Annual Review of Food Science and Technology, 10, 241-259. doi: 10.1146/annurev-food-032818-121301.

Guenther, S., Huws, S., & Huws, L. (2020). Role of Enterobacteriaceae in human foodborne infections. International Journal of Environmental Research and Public Health, 17(20), 7417.

Rajal, V. B., Alcaráz, L. E., & Farber, J. M. (2005). A comparison of alternative solid supports for the enumeration of foodborne pathogens using the polymerase chain reaction (PCR) and Petrifilm(TM). Journal of Food Protection, 68(3), 536-540.

Silbernagel, K. M., & Lindberg, K. G. (2003). 3M Petrifilm enterobacteriaceae count plate method for enumeration of enterobacteriaceae in selected foods: collaborative study. Journal of AOAC International, 86(4), 802–814. <https://doi.org/10.1093/jaoac/86.4.802>

Silbernagel, K. M., & Lindberg, K. G. (2002). Evaluation of the 3M petrifilm Enterobacteriaceae count plate method for the enumeration of Enterobacteriaceae in foods. Journal of Food Protection, 65(9), 1452–1456. https://doi.org/10.4315/0362-028x-65.9.1452

3M Enterobacteriaceae count plates. (n.d.). Safefoodltd.com. Retrieved September 18, 2023, from https://safefoodltd.com/3M-Enterobacteriaceae-Count-Plates

Abgrall, B., & Cleret, J. J. (1990). Evaluation of petrifilmTM SM for the enumeration of the aerobic flora of fish. Journal of Food Protection, 53(3), 213–216. https://doi.org/10.4315/0362-028x-53.3.213

Medina, L. M., & Jordano, R. (2014). Petrifilm – A Simplified Cultural Technique. In Encyclopedia of Food Microbiology (pp. 19–24). Elsevier.

Drebes, T., Majolo, C., & Fröder, H. (2012). Evaluation of the use of PetrifilmTM EB count plates for the enumeration of Enterobacteriaceae in poultry samples. Food Science and Technology, 32(3), 588-593. <https://doi.org/10.1590/s0101-20612012005000088>

Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A., & Uyttendaele, M. (2010). Alternative microbial methods: An overview and selection criteria. Food Microbiology, 27(6), 710–730. https://doi.org/10.1016/j.fm.2010.04.008

Feng, G., Hew, A., Manoharan, R., & Subramanian, S. (2017). Impact of mannanase-producing Bacillus spp. On the accuracy of the 3M petrifilm aerobic count method. Journal of Food Protection, 80(7), 1117–1122. https://doi.org/10.4315/0362-028x.jfp-16-473

Basak, S., & Shetty, P. H. (2021). Conventional microbial counting and identification techniques. In Techniques to Measure Food Safety and Quality (pp. 69–89). Springer International Publishing.

Biesta-Peters, E. G., Kinders, S. M., & de Boer, E. (2019). Validation by an interlaboratory collaborative trial of EN ISO 21528 - microbiology of the food chain - horizontal methods for the detection and enumeration of Enterobacteriaceae. International Journal of Food Microbiology, 288, 75–81. https://doi.org/10.1016/j.ijfoodmicro.2018.05.006

Vasavada, P. C., Lee, A., & Betts, R. (2020). Conventional and novel rapid methods for detection and enumeration of microorganisms. In Food Engineering Series (pp. 85–128). Springer International Publishing.