Increased Productivity with Neogen® Petrifilm® Plates

Introduction

Recent labor shortages have resulted in having to carry equivalent or disproportionate workloads with fewer people in food testing laboratories.

Even in times when labor isn't scarce, efficiency is valued in laboratories as regulatory or accreditation requirements necessitate clear documentation, data tracking, personnel training, implementation of new monitoring procedures or audits. Routine microbiological testing is one area that can be of focus to increase efficiency by reducing the time needed to prepare media and shortening the time in which results are received.

In a study conducted in 2021–22, three contract laboratories ran a side-by-side study comparing the time spent to perform reference methods using traditional media to rapid, alternative methods with Neogen Petrifilm Rapid Plates in conjunction with the Neogen Petrifilm Plate Reader Advanced. Neogen Petrifilm Rapid Plates are a sample-ready culture-medium-system which contain nutrients, a cold-water-soluble gelling agent, and indicator dyes to facilitate enumeration. The Neogen Petrifilm Plate Reader Advanced is a compact instrument that automates the enumeration of eleven different Neogen Petrifilm Plates to enhance laboratory productivity and ensure accurate, consistent readings. Results are stored in the Neogen Petrifilm Plate Manager Software for data analysis.

The intention of the study was to compare the two testing processes for efficiency of method and technician time. Time was measured from preparation of media to disposal for samples within three food categories: Non-fluid dairy (e.g. cheese, ice cream), ready-to-eat foods, and meat samples. The methodologies in scope for this evaluation are reference methods and Neogen Petrifilm Rapid Plate methods. The methods used to evaluate food samples in this study included enumeration of total aerobic count (also known as total plate count or standard plate count), *E. coli*, coliforms and yeast and mold. The performance of the methods was out of scope for this study.

Goal of the Study

1. Compare hands-on test time for the technician
2. Compare efficiency of method

Materials and Methods

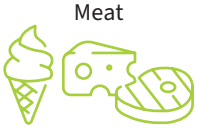
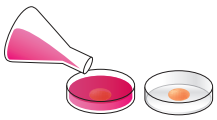
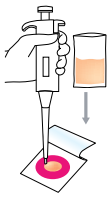
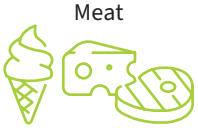
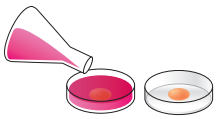
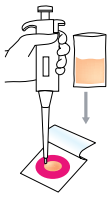
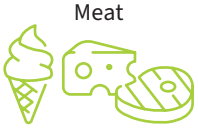
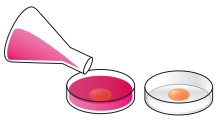
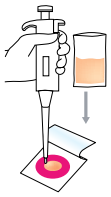
Media and Reagents

- Reference Method Media and Materials
- Plate Count Agar
- Violet Red Bile Lactose Agar with 4-Methyl-umbelliferyl-ß-D-glucuronide (MUG)
- Lauryl Tryptose Broth
- Brilliant Green Lactose Bile Broth
- EC Broth
- Violet Red Bile Lactose Agar
- Potato Dextrose Agar
- Dichloran Rose Bengal Chloramphenicol Agar
- Tryptone Bile X-Glucuronide (TBX) Agar
- Neogen Petrifilm Rapid Aerobic Count Plate
- Neogen Petrifilm Rapid Coliform Count Plate
- Neogen Petrifilm Rapid *E. coli*/Coliform Count Plate
- Neogen Petrifilm Rapid Yeast and Mold Count Plate
- Neogen Petrifilm Flat Spreader
- Neogen Petrifilm Spreader
- Stomacher Bags
- Homogenizer
- Matrices: Dairy Products, Meat Products and Ready-to-Eat Products
- Incubators
- Appropriate Sterile Diluents

The study took place across three laboratories based in the United States which were proficient in the methods used (Table 2 and Table 3). The laboratories purchased a range of samples across three food categories: non-fluid dairy products, ready-to-eat foods and meat products. Samples were split into two portions prior to the start of the study.

Over the course of three test days, with varying numbers of samples each day, a total of 105 samples were tested. On test day one, fifteen samples were tested with a combination of the three sample types, with a minimum of 25% of samples from each category. The same combination of three sample types, with a minimum of 25% of samples from each food category were tested on test days two and three with 30 and 60 samples tested respectively. On test days one and two, one analyst completed the testing using only Neogen Petrifilm Plate methods and a second analyst tested the samples following a traditional media-based reference method. Test day three allowed for two analysts to conduct the Neogen Petrifilm Plate methods and two analysts for the reference methods. These analysts performed the testing in a side-by-side manner using the same sample sets split into two portions.

Table 1: Study Protocol Deployed in Three Laboratories

Day	Sample #	Sample Mix	Method 1	Method 2
Day 1	15	≥ 25% Non-fluid dairy Cheese Meat 	 **Analyst 1** conducts traditional method 	 **Analyst 2** conducts Petrifilm Plate method 
Day 2	30	≥ 25% Non-fluid dairy Cheese Meat 	 **Analyst 2** conducts traditional method 	 **Analyst 1** conducts Petrifilm Plate method 
Day 3	60	≥ 25% Non-fluid dairy Cheese Meat 	 **Analyst 1 and Analyst 2** conduct traditional method 	 **Analyst 3 and Analyst 4** conduct Petrifilm Plate method 

One of the laboratories followed ISO Standard methods for the reference method testing. The ISO standards used, and the tests performed for each of the food categories are listed in Table 2. Confirmation testing was included as part of the study as described in the relevant standards.

Table 2: Test Methods Performed Comparing ISO Standard Methods to Neogen Petrifilm Rapid Plates

Food Category	Reference Method	Neogen Petrifilm Rapid Plate Method
Non-fluid dairy products 	ISO 4833 -1:2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique	Petrifilm Rapid Aerobic Count Plate
	ISO 4831:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique	Petrifilm Rapid Coliform Count Plate
	ISO 21527-1: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and molds — Colony count technique in products with activity greater than 0.95	Petrifilm Rapid Yeast and Mold Count Plate
Ready-to-eat products 	ISO 4833 -1:2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique	Petrifilm Rapid Aerobic Count Plate
	ISO 16649-2: 2001. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*	Petrifilm Rapid *E. coli*/Coliform Count Plate
	ISO 21527-1: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and molds — Colony count technique in products with activity greater than 0.95	Petrifilm Rapid Yeast and Mold Count Plate
Meat products 	ISO 4833 -1:2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique	Petrifilm Rapid Aerobic Count Plate
	ISO 16649-2: 2001. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*	Petrifilm Rapid *E. coli*/Coliform Count Plate

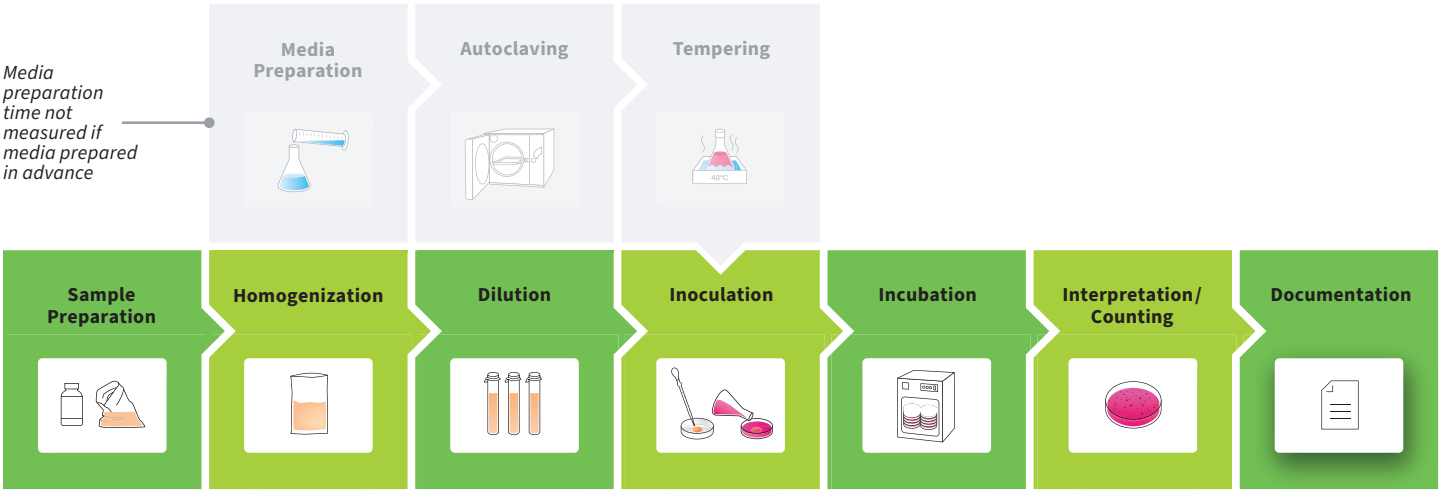
The remaining two laboratories followed the United States Food and Drug Administration (FDA) and Bacteriological Analytical Manual (BAM) for the reference method testing. The methods used, and the tests performed for each of the food categories are listed in Table 3. Confirmation testing was included as part of the study as described in the relevant methods.

Table 3: Test Methods Performed Comparing FDA Bacteriological Analytical Manual Methods to Neogen Petrifilm Rapid Plates

Food Category	Reference Method	Neogen Petrifilm Rapid Plate Method
Non-fluid dairy products 	• FDA BAM Chapter 3, Aerobic Plate Count (32°C incubation)	Petrifilm Rapid Aerobic Count Plate
	• FDA BAM Chapter 4, Enumeration of *Escherichia coli* and the Coliform Bacteria (32°C incubation) — MPN	Petrifilm Rapid Coliform Count Plate
	• FDA BAM Chapter 18, Yeasts, Molds, and Mycotoxins	Petrifilm Rapid Yeast and Mold Count Plate
Ready-to-eat products 	• FDA BAM Chapter 3, Aerobic Plate Count	Petrifilm Rapid Aerobic Count Plate
	• FDA BAM Chapter 4, Enumeration of *Escherichia coli* and the Coliform Bacteria — Solid medium	Petrifilm Rapid *E. coli*/Coliform Count Plate
	• FDA BAM Chapter 18, Yeasts, Molds, and Mycotoxins	Petrifilm Rapid Yeast and Mold Count Plate
Meat products 	• FDA BAM Chapter 3, Aerobic Plate Count	Petrifilm Rapid Aerobic Count Plate
	• FDA BAM Chapter 4, Enumeration of *Escherichia coli* and the Coliform Bacteria – Solid medium	Petrifilm Rapid *E. coli*/Coliform Count Plate

Timing began at the start of the media preparation process (gathering materials) for the reference methods and continued until the end of each test day's method completion including confirmation testing if performed. Time taken for quality assurance testing of reference media was not included in the study. If media was stored after preparation but prior to use, this time was also not recorded. For Neogen Petrifilm Rapid Plate testing, the timing started when plates were taken out of the refrigerator and allowed to come to room temperature and ended once the test days plates had been enumerated and recorded by the Neogen Petrifilm Plate Reader Advanced. The start and stop time for steps included in Figure 1 were recorded to differentiate between active time and passive time.

Figure 1. Steps for Start and Stop Timing for Reference Method and Neogen Petrifilm Plate Method



Sample preparation was conducted following each laboratory's internal procedures for the samples tested. Two of the laboratories (one following ISO Standard methods and the second following FDA methods) diluted and plated all samples at 1:10, 1:100 and 1:1000. The remaining laboratory diluted and plated all samples at 1:10 and 1:100. Plates and MPN tubes were placed into incubators and incubated according to the appropriate incubation time and temperature (Tables 4 and 5). When the method incubation time was complete, agar and MPN tubes were enumerated according to the reference method. Neogen Petrifilm Rapid Plates were enumerated using the Neogen Petrifilm Plate Reader Advanced. All results were recorded, and timing ended once the last of the test days plates were read or confirmation testing was completed.

Table 4: Incubation Conditions for Neogen Petrifilm Rapid Plates Following ISO 16140-2:2016 Validated Methods

Food Category	Neogen Petrifilm Rapid Plate Method	Incubation Temperature	Incubation Time
Non-fluid dairy products 	Petrifilm Rapid Aerobic Count Plate	30 ± 1°C	28 ± 2 hours
	Petrifilm Rapid Coliform Count Plate	35 ± 1°C	24 ± 2 hours
	Petrifilm Rapid Yeast and Mold Count Plate	25 ± 1°C or 28 ± 1°C	60 to 72 hours
Ready-to-eat products 	Petrifilm Rapid Aerobic Count Plate	30 ± 1°C	28 ± 2 hours
	Petrifilm Rapid *E. coli*/Coliform Count Plate	42 ± 1°C	18 to 24 hours
	Petrifilm Rapid Yeast and Mold Count Plate	25 ± 1°C or 28 ± 1°C	60 to 72 hours
Meat products 	Petrifilm Rapid Aerobic Count Plate	30 ± 1°C	28 ± 2 hours
	Petrifilm Rapid *E. coli*/ColiformCount Plate	42 ± 1°C	18 to 24 hours

Table 5: Incubation Conditions for Neogen Petrifilm Rapid Plates following *Official Methods of Analysis*℠ (OMA) of *AOAC INTERNATIONAL*®

Food Category	Neogen Petrifilm Rapid Plate Method	Incubation Temperature	Incubation Time
Non-fluid dairy products 	Petrifilm Rapid Aerobic Count Plate	32 ± 1°C	24 ± 2 hours
	Petrifilm Rapid Coliform Count Plate	35 ± 1°C	24 ± 2 hours
	Petrifilm Rapid Yeast and Mold Count Plate	28 ± 1°C	48 to 60 hours
Ready-to-eat products 	Petrifilm Rapid Aerobic Count Plate	35 ± 1°C	24 ± 2 hours
	Petrifilm Rapid *E. coli*/Coliform Count Plate	35 ± 1°C	18 to 24 hours
	Petrifilm Rapid Yeast and Mold Count Plate	28 ± 1°C	48 to 60 hours
Meat products 	Petrifilm Rapid Aerobic Count Plate	35 ± 1°C	24 ± 2 hours
	Petrifilm Rapid *E. coli*/ColiformCount Plate	35 ± 1°C	18 to 24 hours

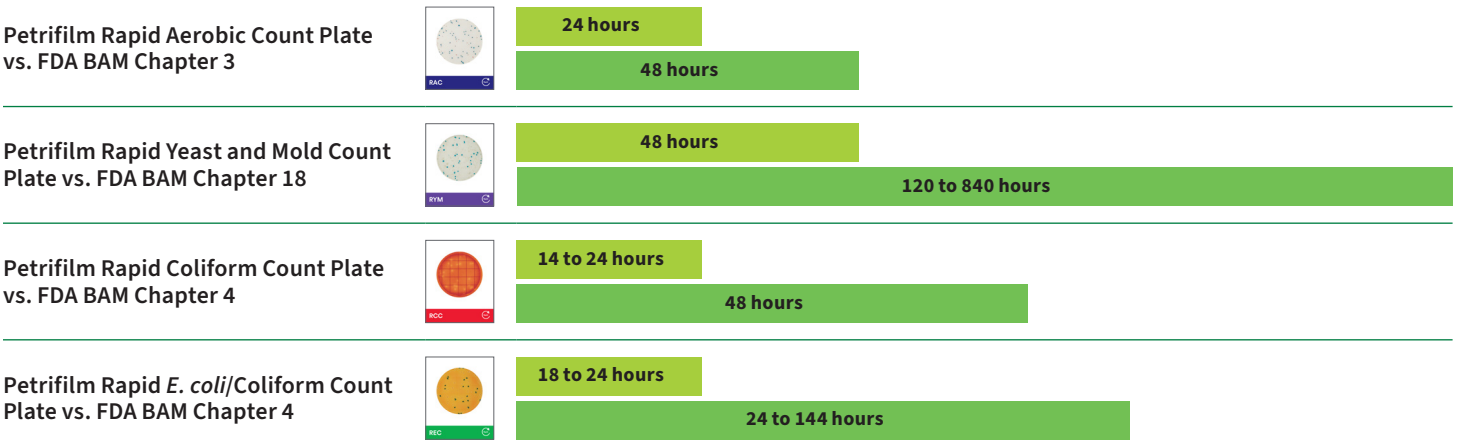
Results and Discussion

Compared to reference methods, Neogen Petrifilm Plates and the Neogen Petrifilm Plate Reader Advanced, demonstrated efficiency and productivity gains in both hands-on technician time and overall method test time. All comparisons showed efficiency gains regardless of sample type and the number of tests performed.

Summary of Results

Compared to reference methods, running the Neogen Petrifilm Rapid Plate methods with the Neogen Petrifilm Plate Reader Advanced demonstrated a savings of 3.5 to 4.3 hours per day of hands-on time** for technicians and the total test time* was reduced by 57% to 63%.

Method Time to Result

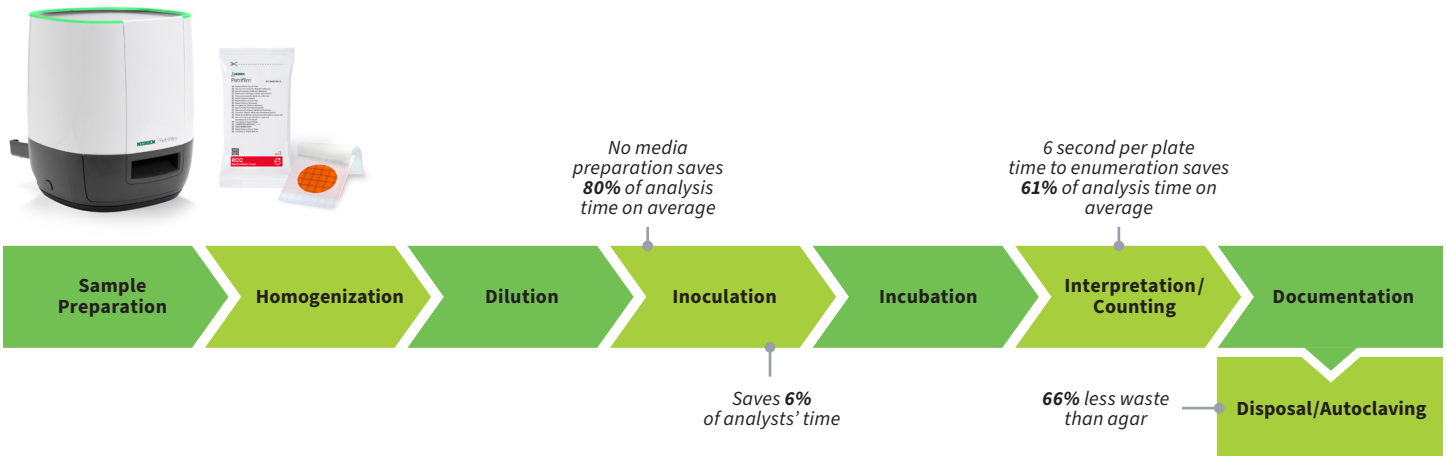


Compare Hands-on Test Time For the Technician

Compared to reference methods, Neogen Petrifilm Plates and the Neogen Petrifilm Plate Reader Advanced reduce the amount of active or hands-on test time an average of 48% or 3.8 hours per 8 hours of test time.**

Compare Efficiency of Method

On average, compared to reference methods, Neogen Petrifilm Plates reduce the amount of active or hands-on test time during media preparation by 80%, the plating process by 6% and the enumeration step using the Petrifilm Plate Reader advanced by 61%.*



*Total testing time includes hands on and test incubation time

**Excludes passive time. Passive time would include incubation time and autoclave cycles.

References

1. Neogen Petrifilm Rapid Yeast and Mold Count Plate 6475/6477 Product Instructions.
2. Neogen Petrifilm Rapid Aerobic Count Plate 6478/6479 Product Instructions.
3. Neogen Petrifilm Rapid *E. coli*/Coliform Count Plate 6436/6437 Product Instructions.
4. Neogen Petrifilm Rapid Coliform Count Plate 6402/6412 Product Instructions.
5. ISO 4833-1 (2013). Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: colony-count technique at 30°C by the pour plate technique.
6. ISO 21527. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and molds — Part 1: Colony count technique in products with water activity greater than 0.95 — Part 2: Colony count technique in products with water activity less than or equal to 0.95.
7. ISO 4831. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique.
8. ISO 16649-2. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* — Part 2: Colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.
9. United States Food and Drug Administration Bacteriological Analytical Manual.

Acknowledgements

Neogen would like to thank Certified Lab (formerly known as Food Safety Net Services), Mérieux NutriSciences, and Q Laboratories for their time to conduct this study.

NEOGEN®

Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 USA.