

# Using Petrifilm in Student Microbiology Studies



*“a world of learning”*

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## Introduction

Microbiology involves working with organisms that are too small to see in normal circumstances. As a result, special techniques, methods and apparatus are usually required. In particular, special agar gel substrates are often employed to grow microorganisms. This workshop presents Petrifilm, an alternative growth substrate made by 3M Corporation.

## What is Petrifilm?

A Petrifilm plate is a thin film, sample ready, dehydrated version of the conventional Petri dish agar plate. Petrifilm is widely used in the food industry throughout the world to monitor quality and to audit cleaning and sterilisation processes. Foods, beverages and surfaces can be tested for the presence of harmful bacteria (pathogens), indicator bacteria (that indicate the possible presence of pathogens), and spoilage organisms that can affect the shelf-life.

The advantages of Petrifilm include:

- Ready to use – no preparation required
- Long shelf life when shielded from moisture and humidity
- Simple to use and interpret
- Built-in biochemical confirmation
- Smaller (thinner) and less likely to be damaged than agar plates.

## Types of Petrifilm

There are many types of Petrifilm, each formulated and designed for a specific purpose. Of the total number available, four are suggested for use in education. Other types that are used in industry to isolate known pathogens are regarded as unsuitable for use by students.

### **Aerobic Count (AC) Plate**

Designed to enumerate common aerobic bacteria. The AC plate contains Standard Methods nutrients, a cold water gelling agent, and triphenyl tetrazolium chloride (TCC), an indicator that colours bacterial colonies red.

## **Coliform Count (CC) Plate**

Designed to enumerate coliform bacteria. Coliforms are members of the family *Enterobacteriaceae* which ferment lactose to produce gas. Coliforms are an indicator of faecal contamination, particularly in dairy products. The CC plate contains violet red bile (VRB) lactose nutrients, a cold water gelling agent and triphenyl tetrazolium chloride (TTC), an indicator that colours bacterial colonies red.

## ***E.coli*/Coliform Count (EC) Plate**

Counts all coliforms in a sample and differentiates *E. coli* as a subset of the total. The EC count is an indicator of faecal contamination, particularly in meat products. The EC plate is essentially the same as the CC plate, except that it contains the BCIG chromogenic indicator.

## **Yeast and Mould Count (YM) Plate**

Designed to count all common yeast and mould species in a sample. The YM plate contains modified Sabroude's Dextrose nutrients in a cold water gelling agent, a dye to enhance the visualization of growth on the plate (colours all yeasts aqua green), and two broad spectrum antibiotics to suppress bacterial growth.

## **Using Petrifilm**

Petrifilm is supplied in sealed packs that should be kept cool and dry. Storage in the dry atmosphere of a freezer is recommended, but ensure you allow the pack to warm up to room temperature before opening to avoid condensation forming.

Take out just the plates you will need for a piece of work, then reseal the pack and put it back in the freezer.

For detailed descriptions of sample preparation, inoculation methods, incubation conditions and interpretation of results, visit [www.southernbiological.com](http://www.southernbiological.com) then follow the links: Catalogue - Kits & Equipment - Petrifilm - Petrifilm Plates and Accessories. From here, you can click through each product code for access to detailed information.

## **Testing Liquids**

Liquids such as orange juice, milk or water can be tested directly by inoculating the Petrifilm plate with 1mL and incubating. If the count is too high, you should dilute the sample then correct for the dilution factor when expressing the result. For information on serial dilution, download the "Tips on Sample Preparation – Serial Dilution" sheet from our website.

Testing potable water can be problematic because standards require no *E.coli* per 100mL of water. In practical terms, this means you would have to perform 100 tests with EC plates because each plate uses only 1mL of water. It is possible to use a sterile filtration system to concentrate a volume of water for testing, but for student demonstrations, it is more usual to dose a water sample with an *E.coli* culture to ensure a positive result.

## Testing Solids

Solids need to be macerated in sterile liquid to extract microorganisms before they can be tested, but once this has been done, the plate can be inoculated with 1mL of the extract. For detailed instructions, download the "Coliform Information Insert" sheet from our website (follow links from product code M6410).

## Testing Surfaces

Two approaches are possible when using Petrifilm to test for the presence of microorganisms on surfaces. One method is to swab an area, and then to extract the swab with sterile liquid that can be tested. This is useful when testing irregular surfaces such as a tap, door handle, light switch or hand. The 3M Quick Swab (M6432) is a self-contained ready-to-use surface swab system, otherwise you can use a sterile cotton wool or rayon swab held with sterile forceps.

The second method is direct contact with a Petrifilm plate. This is suitable for smooth regular surfaces such as bench tops and floors, and even the surface of the skin. In order to activate the plates, they need to be hydrated with 1mL of sterile liquid before contact. Hydrated plates should be allowed to gel for at least one hour, but can be stored under refrigeration for up to a week before being used to test a surface. For more information, visit our website.

## Testing the Atmosphere

By taking a hydrated plate and peeling back the protective film, you can expose the activated surface of the plate to the air to check for the presence of airborne microorganisms. For example, compare different air conditioner outlets. For a description of the experimental method, download the "Yeast & Mould Information Insert" sheet from our website (follow links from product code M6407).

## Student Experiments

Petrifilm plates are suitable for many student experiments because of their ease of use and versatility. As well as meeting outcomes in the field of microbiology, the use of Petrifilm touches on areas such as maths (dilution and correction factors, calculations, scaling and statistics), scientific experimental design, and use of laboratory equipment. Download a copy of the "Petrifilm Experiment Manual" from our website (follow the link from any of the product codes).

## Accuracy and Precision

Microbiology is a branch of science that is concerned with accuracy but not so much with precision. Even though Petrifilm plates are designed for counting and enumeration of microorganisms, it is usual to speak in terms of "tens" or "hundreds" rather than giving precise numbers such as "47" or "163". All Petrifilm plates are calibrated to receive "1mL", so it is acceptable to use plastic Pasteur pipettes, and there is no need to discard plates that might have a slightly irregular gel shape.

## **Sterile Technique**

All microbiological procedures require sterile methods to avoid contamination that would interfere with the determinations being made. Basic measures include:

- Wash hands thoroughly before and after each procedure.
- Tie back hair.
- Swab the work surface with 70% ethanol before starting work. Leave wet and allow to evaporate dry.
- Use a Bunsen flame to form a heat curtain to shield the work area from airborne microorganisms.
- Use “economical” movements and minimize the time that surfaces and samples are exposed.

When mixing or diluting samples, it is acceptable to use well boiled water that has been allowed to cool. For macerating food samples, you can get good results in a fresh polythene lunch bag. Provided they haven't been opened, these bags can generally be considered as sterile for the purpose of school experiments.

To check for the presence of a background level of microorganisms in the water and/or bag, run a control that substitutes sterile liquid for the test substance.

## **Safety and Disposal**

Following inoculation, plates should be taped shut or placed in a press-seal bag to keep them isolated. Follow good laboratory practice and have students thoroughly wash their hands after handling microbiological samples and equipment. Adequate antibacterial hand wash and hand rub sanitizer solutions should be provided.

Treat plates with viable colonies as you would deal with cultures on conventional Petri dishes. Autoclave or soak in a suitable biocide, e.g. 10% bleach solution. Alternatively, you can use a contract collection service such as that provided by Stericorp.

## **Trouble Shooting**

The most common problems we have seen tend to occur with novice users of Petrifilm, but they are easily avoided:

### ***Dispensing more than 1mL of liquid***

If you accidentally draw more than 1mL of liquid into the pipette and dispense it onto the Petrifilm plate, it will overflow off the plate when the spreader is applied. Be careful to apply only 1mL.

### ***Having the liquid roll off the plate as it is being applied***

This can occur if you hold the pipette at a low angle. Place the Petrifilm plate on a flat surface and hold the pipette perpendicular to the surface, in the central region. Dispense the liquid onto the plate with care.

### ***Waiting too long***

Have the plate, spreader and sample to be tested ready, and apply the spreader without delay after dispensing the sample onto the plate. Proper spreading may not occur if you wait too long between dispensing the liquid and applying the spreader.

### ***Trapping bubbles on the plate***

First of all, ensure the sample does not have entrained air bubbles. Allow it to stand if necessary to allow bubbles to clear. As a further measure, use the recommended method to lower the protective film back onto the plate after dispensing the sample. For the AC and YM plates, allow the film to drop back into place. For the CC and EC plates, roll the film gently back into place.

### ***Using the wrong spreader***

Ensure you use the correct spreader. For YM plates, use only the special (larger size) Yeast and Mould Plate Spreader. For AC plates, use the General Purpose Spreader with the ridge side down (smooth side up). For CC and EC plates, use the General Purpose Spreader with the smooth side down (ridge side up).

### ***Getting contamination***

Avoid contamination by planning your work and using good aseptic technique and sterile equipment. This applies to all microbiological work, not just Petrifilm.

### **Further Information**

The key to good results with microbiology experiments is familiarity with the equipment, confidence in the techniques, and respect for the safety aspects. For more information, contact us or attend a hands-on practical workshop session at a forthcoming conference or seminar. See the "*Events*" section of our web site for times and locations.