Petrifilm™ Coliform Count Plates

"a world of learning"

Description

The Petrifilm Coliform Count Plate from 3M is a ready-made culture medium system for the enumeration of coliform bacteria that might be found in food and beverages, or on work surfaces. Petrifilm Coliform Count Plates contain violet red bile (VRB) lactose nutrients, a cold water gelling agent and triphenyl tetrazolium chloride (TTC), an indicator that colours bacterial colonies red.

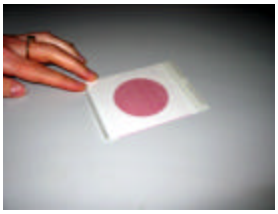

Figure 1

Directions For Use

1. Place the Petrifilm Coliform Count plate on a flat surface (see fig 1).
2. Lift the top film, hold the pipette perpendicular to the plate and carefully dispense 1mL of sample or sterile hydrating solution onto the centre of the bottom film (see fig 2). Sterile hydrating solution is used when you intend to test a surface by direct contact.
3. Roll the top film down onto the liquid, avoiding the entrapment of air bubbles under the top film (see fig 3).
4. Orient the plastic all-purpose spreader with the smooth side down (ridge side up) and place it on the top film over the liquid sample. Press gently on the centre of the spreader to distribute the sample evenly (see fig 4). Avoid sliding or twisting the spreader on the film. Remove the spreader and leave the plate undisturbed for one minute to allow the gel to solidify.
5. Plates treated with sterile hydrating solution should be allowed to gel for at least one hour, but may be stored in a refrigerator for up to 1 week before being used to test a surface.
6. Incubate inoculated plates in a horizontal position (clear side up) at 35°C for 24 hours. Plates may be stacked up to 20 high and placed in a press-seal bag (see fig 5).

Figure 2

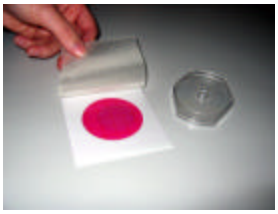

Figure 3

Figure 4

Interpretation

1. Coliform Count plates will indicate all coliforms in the sample. By definition, coliforms are the members of the family Enterobacteriaceae which ferment lactose to produce gas. Count all colonies which appear red and which are associated with gas bubbles as confirmed coliforms.
2. Express the count in terms of the number of coliforms per sample.
3. The presence of many small colonies, many gas bubbles, or an overall deepening of the colour of the gel probably indicates that the sample is too concentrated to give a reliable result. In this case, further dilution of the sample is required to obtain an accurate count.

Storage

Store unopened packs of Petrifilm Coliform Count plates in a freezer. Allow the pack to come to room temperature before opening. After opening and removing the plates you need, the pack may be resealed with tape and/or placed in a press-seal bag and returned immediately to the freezer.

After removal from the pack, plates should be kept in cool dry conditions (below 25°C and 50%RH) and used within one month.

Figure 5

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Petrifilm™ Coliform Count Plates
Suggested Experiment

Coliforms in minced meat.

In this experiment, you will extract a sample of minced meat with sterile diluent, and then estimate the concentration of coliforms in the meat by diluting and plating samples.

Carefully tare a sterile "stomacher" bag or a suitable equivalent on a balance, then add 8 – 12g of minced meat. Use a sterile spoon or spatula to transfer the meat and avoid any contact that might contaminate the sample or the interior of the bag during this process. Record the weight of the meat to the nearest 0.1g (see fig 6). Effect a 10x dilution of the sample by adding 90mL of sterile diluent to the bag, then fold over the top and thoroughly blend the contents by squeezing and macerating with one hand whilst holding the top closed with the other (see fig 7). Tilt the bag to one side and allow the solids to settle in a corner, then carefully withdraw 1mL using a sterile pipette (see fig 8). Inoculate a Coliform Count Plate by dispensing the 1mL sample as described in the instructions overleaf, then incubate at 35°C for 24 hours. Confirmed coliforms will produce red colonies with associated gas bubbles. Red spots without gas bubbles should not be counted as they may be food particles or non-coliform bacteria. Express the result as the number of coliforms per gram of meat after scaling for the volume of diluent and correcting for the difference between the actual weight of the sample and its nominal weight of 10g. For example, if a sample of 9.2g of meat gave a count of 27 coliforms, the concentration of coliforms would be: 27 x 10 x (10/9.2) = 294 coliforms per gram of meat.

After incubation, plates can be sealed in a press-seal bag and frozen for future reference. Alternatively, they can be photographed and disposed of.

Figure 6

Figure 7

Figure 8

Going Further:

- Repeat the experiment after leaving the meat unrefrigerated for several hours.
- Test minced meat obtained from different sources.
- Cook the meat and repeat the test
- Run concurrent tests using Aerobic Count Plates and *E. coli*/Coliform Count Plates to get an idea of the relative populations of different types of bacteria.

Safety and Disposal

Following inoculation, plates presented to the class for examination and counting 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 sanitiser solutions should be provided.

Plates with viable colonies must be disposed of in a responsible way such as by autoclaving or soaking in an appropriate disinfectant. Alternatively, you can use a contract collection service such as that provided by Stericorp.

More Information

To receive a full colour "pdf" version of these notes, please email a request to sales@southernbiological.com.

For further information about Coliform Count plates and other Petrifilm products, please visit the "Catalogue" section of our web site. You'll find more suggestions for student experiments as well as further details on sample preparation, usage techniques and interpretation of results.

To attend a participative hands-on workshop on how to successfully introduce Petrifilm to your school science curriculum, consult the "Events" section of our web site to check times and locations.

In addition, we would welcome a call if you have any remaining questions relating to Petrifilm and its uses.

Acknowledgement

This information was prepared with the generous assistance and cooperation of Mr B Marks of 3M Australia and Dr J Ralph of TAFE South Australia.