3M™ Petrifilm™ Aerobic Count Plates

Introduction

Petrifilm™ plates are designed to determine total aerobic bacterial populations. The easy-to-use Petrifilm plates contain a film coated with nutrients and gelling agents. You will be able to monitor environmental bacterial counts using a variety of methods. Three environmental sampling methods are described as well as the standard inoculation procedure.

Concepts

- Microbiology
- Plate counting

Materials

Petrifilm Aerobic Count Plates
Petrifilm Spreader
Chip clips
Cotton swab, sterile
Incubator
Pipet
Tape, double-sided
Test tube, screwtop

Safety Precautions

Wear chemical-resistant gloves while working with microbial organisms. After use, Petrifilm Aerobic Count Plates will contain viable cultures. Do NOT reopen the Petrifilm plates or handle unnecessarily. Wash hands thoroughly with soap and water before leaving the laboratory. Extreme caution should be exercised when handling culture materials, and students should be trained in aseptic techniques. Always clean work areas and wash hands after working with microbiological materials.

Procedures

Standard Inoculation

1. Place the Petrifilm Aerobic Count Plate on a flat surface. Carefully peel open the Petrifilm plate being careful not to touch the nutrient gel with your fingers. (See Figure 1.)
2. With a pipet perpendicular to the Petrifilm plate, place 1 mL of sample (inoculum) onto the center of the bottom film. (See Figure 2.)
3. Release the top film; allow it to drop. Do *not* roll top film down. (See Figure 3.)

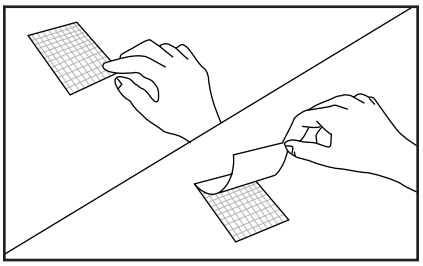

Figure 1.

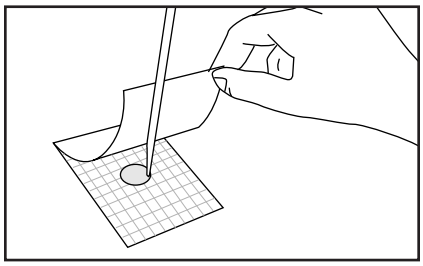

Figure 2.

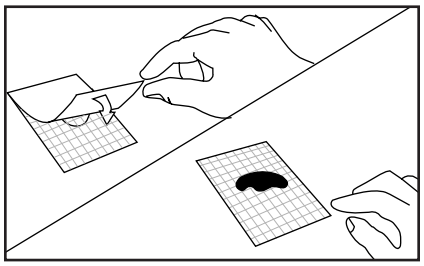

Figure 3.

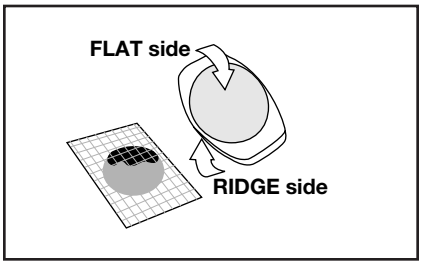

Figure 4.

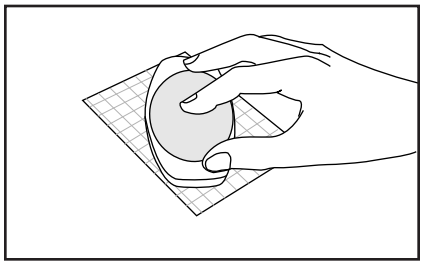

Figure 5.

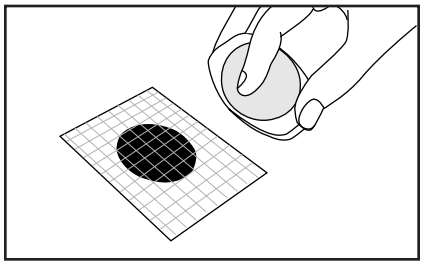

Figure 6.

Publication No. 10163
061616

4. With *ridge* side down, place the spreader on the top film over the inoculum. (See Figure 4.)
5. Gently apply pressure on the spreader to distribute the inoculum over a circular area. Do not twist or slide the spreader, simply apply a gentle downward pressure to the spreader. (See Figure 5.)
6. Lift the spreader. Wait at least one minute for the gel to solidify. (See Figure 6.)
7. Incubate plates with the clear side up in stacks of up to 20. Humidify incubator to minimize loss of moisture. Incubate plates for 48 hours ± 3 hours at 35 °C or 72 hours ± 3 hours at 30 °C ± 1 °C.
8. Plates can now be counted on a standard colony counter or other magnified light source.

Air Sampling Method

Petrifilm plates can be positioned either horizontally or vertically for environmental air sampling.

1. Hydrate the plates using distilled water and following steps 1–5, above, for a standard inoculation. Allow the hydrated plates to remain closed for a minimum of 1 hour before use.
2. For horizontal positioning, use a plastic chip clip in combination with double-sided tape. Position the hinged edge of the Petrifilm plate into the clip. Apply a small piece of double-sided tape to each end of the clip handle. (See Figure 7.)
3. Without touching the circular growth area, lift the top film portion of the hydrated plate and peel it back until the outer portion adheres to the double-sided tape. (See Figure 8.)
4. Expose the Petrifilm plate surface to air for no longer than 15 minutes. Remove the film from the clip, refold, incubate, and examine for microbes.
5. Double-stick tape can be used with or without the plastic clip for vertical positioning of the Petrifilm for air sampling. (See Figure 9.)

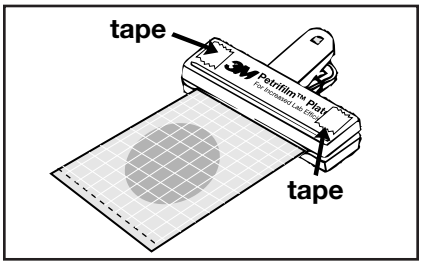

Figure 7.

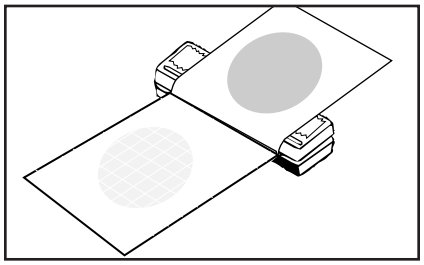
Figure 8.

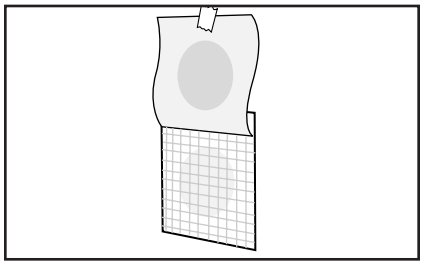
Figure 9.

Swab Contact Method

1. Fill a screw-capped test tube one-half full of distilled water. Autoclave the test tube with the cap loosened. Moisten the sterile cotton swab head in the sterile rinse solution and press out any excess solution against the interior wall with a rotating motion. (See Figure 10.)
2. Locate the surface to be tested. Hold the swab handle to make a 30° angle with the surface. Rub the swab slowly and thoroughly over the desired surface area. Rub the swab three times over the surface, reversing direction between successive strokes. (See Figure 11.)
3. After the area has been swabbed, break off the swab head into the rinse solution and replace cap. Shake the test tube vigorously for 10 seconds. Inoculate the Petrifilm plates with 1 mL of solution following steps 1–5 of the standard inoculation procedure.
4. Inoculate and interpret as described in the standard procedure.

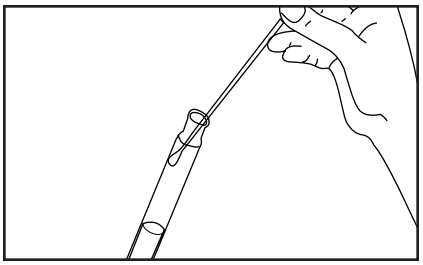

Figure 10.

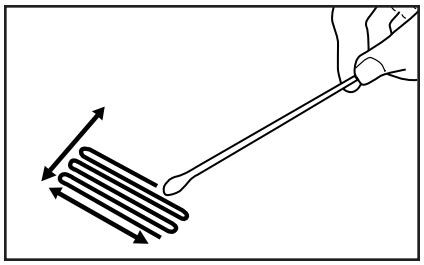

Figure 11.

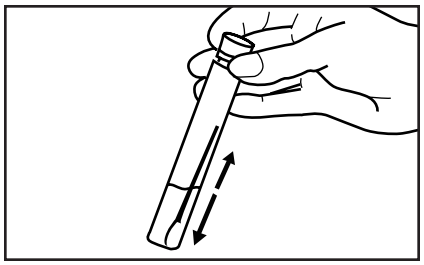

Figure 12.

Direct Contact Method

1. Hydrate the Petrifilm plates following steps 1–5 of the standard procedure.
2. Allow a minimum of 4 hours at room temperature for the gel to solidify on the Petrifilm Aerobic Count Plates.

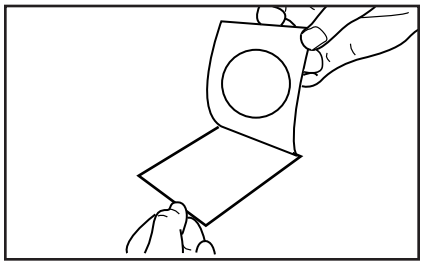

Figure 13.

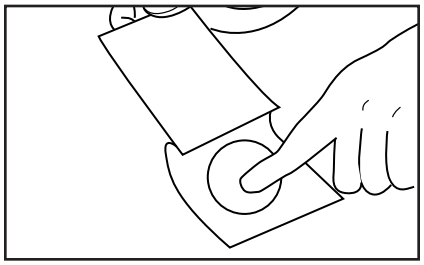

Figure 14.

3. When the gel is solid, carefully lift the top film portion of the hydrated plate. Avoid touching the circular growth area. Gel will adhere to the top film. (See Figure 13.)
4. Allow the circular gel portion of the top film to contact the surface being tested. Rub fingers over the outer film side of the gelled area to ensure good contact with the surface. As with any surface sampling method, the best microbial practice is to wipe the surface after contact with the gel. (See Figure 14.)
5. Lift the film from the test surface and rejoin the top and bottom sheets of the Petrifilm plates.
6. Incubate and interpret following the standard procedures.

Disposal

After use, Petrifilm Aerobic Count Plates will contain viable yeast and/or mold cultures. Do not separate or handle the plates unnecessarily. Treat them using sterile techniques even though the amount of media available for growth is very limited. Opening the plates and soaking them in 10% bleach solution or autoclaving them should precede their disposal in the garbage.

Tips

- Store unopened Petrifilm Aerobic Count Plate foil pouches at or below 46 °F (8 °C).
- After opening, return unused plates to the foil pouch. Seal the pouch by folding and taping the open end.
- Store resealed foil pouches in a cool dry place. Use plates within one month after opening.
- Exposure of Petrifilm Aerobic Count Plates to temperatures and/or humidities above 75 °F (25 °C) and 50% relative humidity can affect the performance of the plates.

Discussion

The Petrifilm Aerobic Count Plates are designed to determine total aerobic bacterial populations. Sampling procedures as outlined in the procedures section allow determinations to be made from nearly any surface as well as from air samples. The convenient, compact, prepared media allow easy access for field sampling in remote areas.

Aerobic Count Plates are great for total aerobic population determination. Incubate for 48 hours for best results. A red indicator dye is in the gelling agent which produces red colonies that provide better contrast for easier colony counting. Count all colonies regardless of size or color intensity. As with agar-pour plates, the preferable counting range on a Petrifilm plate is 25–250 colonies. If greater numbers are achieved, dilution strategies are recommended.

Acknowledgements

Petrifilm materials are a 3M manufactured product and are to be used for non-commercial research and development purposes only.

Materials for *Petrifilm Aerobic Count Plates* are available from Flinn Scientific, Inc.

Catalog No.	Description
FB1171	Petrifilm Aerobic Count Plates
FB1134	Petrifilm Spreader

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.