Project title

Environmental monitoring for quality assurance

Purpose *Why the SOP is needed*	To explore, learn about, and practice the use of 3M Petrifilms and their use in environmental monitoring.
Scope *Who the SOP applies to*	This SOP applies to all laboratory personnel performing the investigation of environmental monitoring/use of petrifilms.
Responsibility *Roles of individuals (e.g., lab technician, principal investigator)*	It is the responsibility of the person(s) performing this process to read and understand this SOP.
References *Cited guidelines, manuals, or literature*	3M Petrifilms background
Principle *Rationale or justification*	Environmental monitoring is a systematic way of testing surfaces, products, and even the air in food and pharmaceutical production facilities, as well as in hospitals and research laboratories. This activity will allow you to practice using one of the items that may be used in these environments, 3M Petrifilm products. There are many different types of this product that will test for many different organisms. The films in this SOP test for *Enerobacteriaceae,* aerobic bacteria, and yeast and mold.
Materials and supplies	• P1000 micropipette • Pipette pump (green) • Stopwatch / timer (phone) • Incubator • Film spreader • Personal protective equipment (PPE): lab coat and gloves • Distilled water • 3M Petrifilms – AB, EB, and YM • P1000 micropipette tips • Serological pipet, 10 mL • Permanent marker • Whirl-Pak • Chip clip • Double-sided tape • Cotton swab • 10% bleach solution • 70% ethanol

Procedure

1. Label the three different Petrifilms with the date, your initials, and the location:
   a. Aerobic bacteria (AB)
   b. *Enterobacteriaceae* (EB)
   c. Yeast and mold (YM)
2. Prepare the *Enterobacteriaceae (*EB) film for use.
   a. Place the Petrifilm plate on a level surface, with the *gridded* side down. Lift the top film. (fig. 1)
   b. With a P1000 micropipette draw 1 mL of distilled water. Holding the micropipette perpendicular to the Petrifilm plate, place 1 mL of distilled water onto the center of the bottom film to hydrate it. (fig. 2)
   c. Roll the top film onto the bottom film. Do not drop the top film down. (fig. 3)
   d. With the *flat* side down, place the film spreader on the top film over the inoculum. (fig. 4)
   e. Gently apply pressure on the spreader to distribute the inoculum over a circular area. Do not twist or slide the spreader. (fig. 5)
   f. Lift the spreader. Leave the film and wait at least 90 minutes for the gel to solidify.
3. Prepare the Aerobic bacteria (AB) Petrifilm for use. The side with the *circular ridge* should be down in step 2d for this procedure. Repeat all of the steps in 2 (a, b, c, d, e, f) on the Aerobic bacteria (AB) Petrifilm.
4. Label the Whirl-Pak with the date, your initials, and the location you sample.
   a. Add 10 mL of distilled water to the Whirl-Pak bag.
   b. Pick the location you want to swab and dip the cotton swab into the Whirl-Pak, then swab a 5 cm × 5 cm square area on the table.
   c. Break off the excess of the cotton swab (so it will fit in the Whirl-Pak) and place the swab into the Whirl-Pak.
   d. Seal the Whirl-Pak bag and homogenize (mix) the contents with your thumb and fingers for 30 seconds.
   e. Using the P1000 micropipette, extract 1 mL of the solution.
   f. Open the YM Petrifilm and place the 1 mL of solution onto the middle of the film.
   g. Slowly lower the cover over the liquid.
   h. Let sit for 1 minute, then use the spreader to apply slight pressure to evenly distribute the solution.
   i. Take film to the incubator and incubate for 24 hours.
5. Go to your workspace and clean and sanitize it with 70% ethanol.
6. Prepare AB Petrifilm to gather sample and incubate.
   a. Position the film so the hinged edge is put into the clip. Next, apply double-sided tape to each end of the clip. (fig. 6)
   b. Without touching the circular growth area, lift the top of the film portion of the hydrated plate and peel it back until the outer portion adheres to the double-sided tape. (fig. 7)
   c. Expose the Petrifilm plate for no longer than 15 minutes. Remove the film from the clip, refold, and place in the incubator for 24 hours.

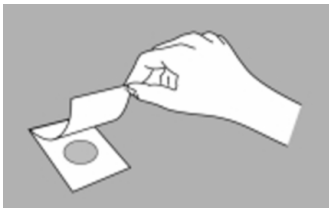


FIGURE 1

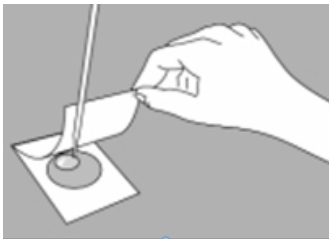


FIGURE 2

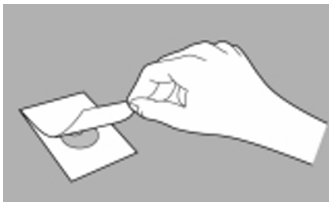


FIGURE 3

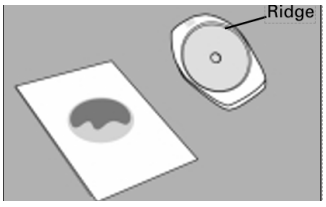


FIGURE 4

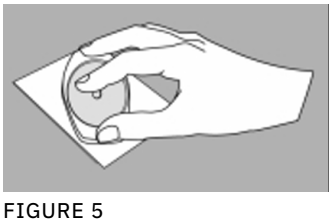


FIGURE 5

7. Prepare EB Petrifilm to gather a sample and incubate.
    a. Carefully lift the top film portion of the hydrated EB plate. *Do not touch the circular growth area.* Gel should adhere to the top of the film. (fig. 8)
    b. Let the circular gel portion of the top film contact the surface being tested (your smart phone screen). (fig. 9)
    c. Lift the film from the test surface and rejoin the top and bottom sheets of the Petrifilm plate. Place in the incubator for 24 hours.
8. Upon returning to the lab the next day, gather your YM, AB, and EB Petrifilms to prepare to analyze.
    a. Retrieve your samples from the incubator.
    b. Use the following interpretation guides for each sample. Your analysis will be recorded on the Data analysis document.
        - ***ols.plus/petrifilm-ab***
        - ***ols.plus/petrifilm-ym***
        - ***ols.plus/petrifilm-eb***
9. Upon completion, open each of your samples, and place your samples in the 10% bleach solution located in the fume hood.
10. Spray and wipe down your work area with 70% ethanol. Dispose of any trash left from your work into the regular garbage and return any other equipment or supplies to the middle of your lab table.
11. Put your lab coat away.
12. Turn in this completed SOP by placing it in the area designated by your lab instructor.

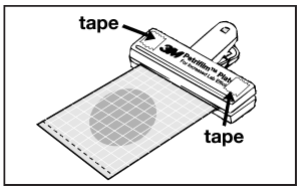
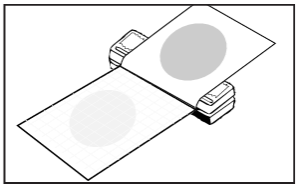


FIGURE 6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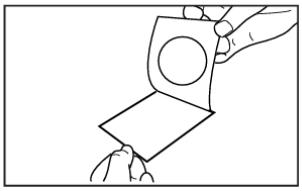
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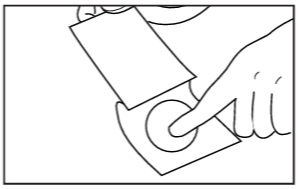
FIGURE 9