3M | Petrifilm™

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Product Instructions

E. coli / Coliform Count Plate

EC

E. coli / Coliform Count

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Issue Date: 2015-08

Product Instructions

E. coli / Coliform Count Plate

Product Description and Intended Use

The 3M™ Petrifilm™ *E. coli* / Coliform Count (EC) Plate is a sample-ready-culture medium system which contains modified Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG), and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm EC Plates are used for the enumeration of *Escherichia coli (E. coli)* and coliforms in the food and beverage industries. 3M Petrifilm EC Plate components are decontaminated though not sterilized. 3M Food Safety is certified to International Standards Organization (ISO) 9001 for design and manufacturing. 3M Petrifilm EC Plate has not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

Safety

The user should read, understand, and follow all safety information in the instructions for 3M Petrifilm EC Plate. Retain the safety instructions for future reference.

⚠ **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

⚠ WARNING

Do not use this plate for the detection of *E. coli* O157. Because most *E. coli* O157 strains are atypical, for example they are glucuronidase negative, they will not produce a blue color, and will not be detected on 3M Petrifilm EC Plates.

To reduce the risks associated with exposure to biohazards and environmental contamination:

- Follow current industry standards and local regulations for disposal of biohazardous waste.

To reduce the risks associated with release of contaminated product:

- Follow all product storage instruction contained in the instructions for use.
- Do not use beyond the expiration date.

To reduce the risks associated with bacterial infection and workplace contamination:

- Perform 3M Petrifilm EC Plate testing in a properly equipped laboratory under the control of a skilled microbiologist.
- The user must train personnel in current proper testing techniques: for example, Good Laboratory Practices[1], ISO 17025[2] or ISO 7218[3].

To reduce the risks associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm EC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm EC Plates for testing water, pharmaceuticals, or cosmetics.
- Do not use 3M Petrifilm EC Plates in the diagnosis of conditions in humans or animals.
- The 3M Petrifilm EC Plates do not differentiate any one microorganism strain from another.

Consult the Safety Data Sheet for additional information.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

Limitation of Warranties / Limited Remedy

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M.

Limitation of 3M Liability

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

Storage

Store unopened 3M Petrifilm EC Plate pouches refrigerated or frozen at temperatures lower than or equal to 8°C (46°F). Just prior to use, allow unopened pouches to come to room temperature before opening. Return unused 3M Petrifilm EC Plates to pouch. Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool dry place for no longer than four weeks. It is recommended that resealed pouches of 3M Petrifilm EC Plates be stored in a freezer (see below) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 50% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm EC Plates in a sealable container. To remove frozen 3M Petrifilm EC Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. 3M Petrifilm EC Plates should not be used past their expiration date. The freezer that is used for open pouch storage must not have an automatic defrost cycle as this would repeatedly expose the plates to moisture which can damage the plates.

Do not use 3M Petrifilm EC Plates that show discoloration. Expiration date and lot number are noted on each package of 3M Petrifilm EC Plates. The lot number is also noted on individual plates 3M Petrifilm EC Plates.

⚠ Disposal

After use, 3M Petrifilm EC Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.

Instructions for Use

Sample Preparation

1. Use appropriate sterile diluents:

   Butterfield's phosphate buffered dilution water[4], 0.1% peptone water, peptone salt diluent, quarter-strength Ringer's solution, saline solution (0.85-0.90%), bisulfite-free letheen broth or distilled water.

   Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm EC Plates; they can inhibit growth. If citrate buffer is indicated in the standard procedure, substitute with one of the buffers listed above, warmed to 40-45°C.
2. Blend or homogenize sample.
3. For optimal growth and recovery of microorganisms, adjust the pH of the sample suspension to 6.6 - 7.2. For acidic products, adjust the pH with 1*N* NaOH. For alkaline products, adjust the pH with 1*N* HCl.

Plating

1. Place the 3M Petrifilm EC Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular to the inoculation area dispense 1 mL of sample suspension onto the center of bottom film.
3. Roll the top film down onto the sample to prevent trapping air bubbles.
4. Place the 3M™ Petrifilm™ Spreader with the flat side down on the center of the plate. Press gently on the center of the 3M Petrifilm Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm EC Plate growth area before the gel is formed. Do not slide the 3M Petrifilm Spreader across the film.
5. Remove the 3M Petrifilm Spreader and leave the 3M Petrifilm EC Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm EC Plates in a horizontal position with the clear side up in stacks of no more than 20 plates. Several incubation times and temperatures can be used depending on current local reference methods, some of which are listed in the "**Specific Instructions for Validated Methods**" section.

Interpretation

1. 3M Petrifilm EC Plates can be counted using a standard colony counter or other illuminated magnifier. Do not count colonies on the foam dam since they are removed from the selective influence of the medium. Do not count artifact bubbles that may be present.

   The interpretation of *E. coli* colonies on the 3M Petrifilm EC Plate is as follows:

   AOAC Official Methods (998.08 and 991.14) – enumerate blue to red-blue colonies associated with entrapped gas, regardless of size or intensity of color, as confirmed *E. coli*. Blue colonies without gas are not counted as *E. coli*.

   Other coliform colonies are red and closely associated (within one colony diameter) with entrapped gas. Colonies not associated with gas (a distance greater than one colony diameter between colony and gas bubble) are not counted as coliforms. The total coliform count consists of both the red and blue colonies associated with gas at 24 hours. Anytime within the validated method incubation period that a blue colony associated with gas appears, it is a confirmed *E. coli*.

⚠ WARNING

Do not use this plate for the detection of *E. coli* O157. Because most *E. coli* O157 strains are atypical, for example they are glucuronidase negative, they will not produce a blue color, and will not be interpreted as *E. coli* on 3M Petrifilm EC Plates.

2. The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.
3. When present in large numbers, 3M Petrifilm EC Plates may have one or more of the following characteristics: a deepening of the gel color with many small, indistinct colonies; and many gas bubbles. High concentrations of *E. coli* will cause the growth area to turn blue, while high concentrations of coliforms (non-*E. coli*) will cause the growth area to turn dark red. When this occurs, record results as too numerous to count (TNTC). When an actual count is required, plate at a higher dilution.
4. Where necessary, colonies may be isolated for further identification. Lift the top film using proper testing technique and pick the colony from the gel. Test using standard procedures.
5. If the plates cannot be counted within 1 hour of removal from the incubator, they may be stored for later enumeration by freezing in a sealable container at temperatures lower than or equal to negative 15°C for no longer than one week.

Specific Instructions for Validated Methods

AOAC® Official Methods℠ (998.08 Confirmed *Escherichia coli* Counts in Poultry, Meats and Seafood, Dry Rehydratable Film Method)

Incubate 3M Petrifilm EC Plates 24 hours ± 2 hours at 35°C ± 1°C.

AOAC® Official Methods℠ (991.14 Coliform and *Escherichia coli* Counts in Foods, Dry Rehydratable Film Methods)

For coliform results incubate 3M Petrifilm EC Plates 24 hours ± 2 hours at 35°C ± 1°C.

For *E. coli* results incubate 3M Petrifilm EC Plates an additional 24 hours ± 2 hours (48 hours ± 4 hours total) at 35°C ± 1°C.

References

1. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
2. ISO/IED 17025. General requirements for the competence of testing and calibration laboratories.
3. ISO 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.
4. FDA. Bacteriological Analytical Manual (BAM), 8th Edition, Revision A, 1998.
5. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

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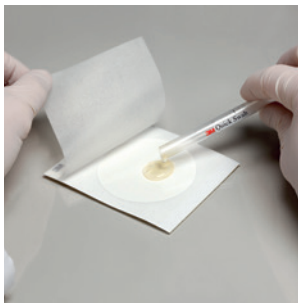
Environmental Monitoring Procedures

3M™ Petrifilm™ Plates are a convenient and reliable way to detect environmental microbial contamination. The construction of 3M Petrifilm Plates allows them to be used for direct contact or swab contact monitoring procedures, as well as air sampling procedures.

Air Sampling

Surface Contact



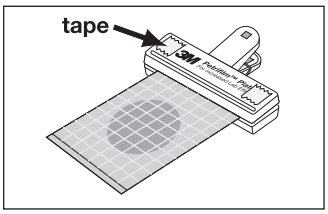
Swabbing

Hydration Procedures for Air or Direct Contact Methods

3M Petrifilm Plate	Hydration*	Storage
Aerobic Count Coliform Count *E. coli*/Coliform Count Rapid Coliform Count *Enterobacteriaceae* Count Yeast and Mold Count Rapid Yeast and Mold Count Rapid *E. coli*/Coliform Count	Hydrate plates with 1 mL of appropriate sterile diluent for a minimum of 1 hour before use. Allow hydrated plates to remain closed for a minimum of 1 hour before use.	Store all hydrated 3M Petrifilm Plates in sealed pouch or plastic bag. Protect plates from light and refrigerate at 2–8°C (36–46°F). Hydrated 3M Petrifilm Aerobic Count Plates may be refrigerated up to 14 days, 3M Petrifilm Rapid Yeast and Mold Count Plates may be refrigerated up to 1 day (24 hours) and all other hydrated 3M Petrifilm Plates may be refrigerated up to 7 days.
Staph Express System	Hydrate plates with 1 mL of appropriate sterile diluent. Refrigerate hydrated plates at 2-8°C (36-36°F) for a minimum of 3 hours before use.	
Rapid Aerobic Count	Hydrate plates with 1 mL of appropriate sterile diluent. For air sampling, refrigerate at 2-8°C (36-46°F) for a minimum of 1 day (24 hours) before use. For direct contact samples, refrigerate at 2-8° C (36-46°F) for a minimum of 3 days before use.	

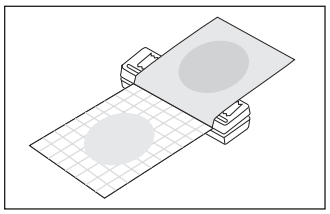
*See relevant 3M Petrifilm Plate product instructions for details and listing of appropriate diluents. If sanitizers are present, use letheen broth for both the direct contact and swab contact methods.

3M Petrifilm Plate Air Sampling Method

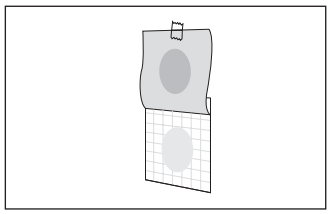


1 Use a 3M Petrifilm Plate clip in combination with double-sided tape. Position hinged edge of hydrated 3M Petrifilm Plate into clip. Apply a small piece of double-sided tape to each end of the clip handle.

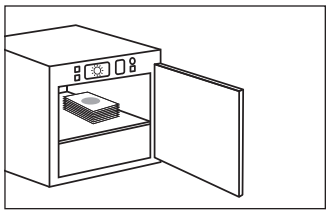
Double-sided tape can also be used with or without clip for positioning of 3M Petrifilm Plates for air sampling.



2 **Without touching circular growth area,** lift top film portion of hydrated plate and peel back until outer portion of film adheres to the tape. Make sure top film lies flat across clip.



3 Expose 3M Petrifilm Plate to air for no longer than 15 minutes. Remove tape and rejoin the top and bottom films.



4 Incubate and enumerate as directed in product instructions. Refer to 3M Petrifilm Plate Interpretation Guide when enumerating results.

Air Sampling Method Results

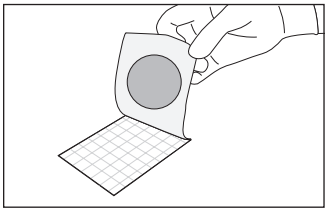
3M Petrifilm Plates: Aerobic Count, Coliform Count, *E. coli*/Coliform Count, Rapid Coliform Count, *Enterobacteriaceae* Count

Results: count/40 cm^2

3M Petrifilm Plates: Staph Express Count, Yeast and Mold Count, Rapid Yeast and Mold Count, Rapid Aerobic Count, Rapid *E. coli*/Coliform Count

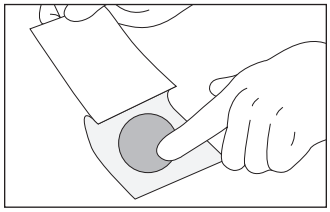
Results: count/60 cm^2

3M Petrifilm Plate Direct Contact Method

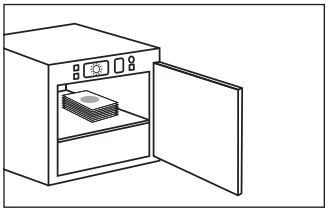


1 Using a hydrated 3M Petrifilm Plate, carefully lift top film. Avoid touching circular growth area. Gel will adhere to top film.

3M™ Petrifilm™ Yeast and Mold Count Plates: On occasion, the gel may split (adhering to both the top and bottom films) when the top film is lifted. If this happens, the plate with gel splitting may still be used for air testing, but is not recommended for direct contact use.



2 Allow the circular gel portion of the top film to contact the surface being tested. Gently rub fingers parallel to the surface over the outer film side of the gelled area to ensure good contact with surface. Rejoin the top and bottom films.



3 Incubate and enumerate as directed in product instructions. Refer to 3M Petrifilm Plate Interpretation Guide when enumerating results.

Direct Contact Method Results

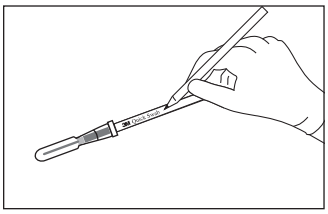
3M Petrifilm Plates: Aerobic Count, Coliform Count, *E. coli*/Coliform Count, Rapid Coliform Count, *Enterobacteriaceae* Count

Results: count/20 cm^2

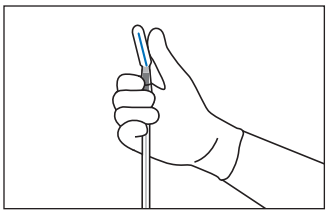
3M Petrifilm Plates: Staph Express Count, Yeast and Mold Count, Rapid Yeast and Mold Count, Rapid Aerobic Count, Rapid *E. coli*/Coliform Count

Results: count/30 cm^2

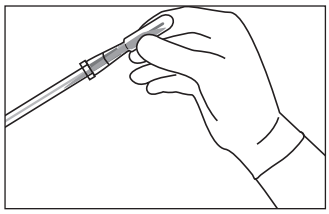
3M™ Quick Swab Method (wet swabbing method)*



1 Remove the desired quantity of 3M Quick Swabs from the resealable plastic bag. Label the swab.



2 At the sampling location, prepare the swab by holding it with the bulb end near your thumb. Bend the red snap valve at a 45° angle until you hear the valve break. This allows the letheen broth to flow into the tube and wet the swab head.

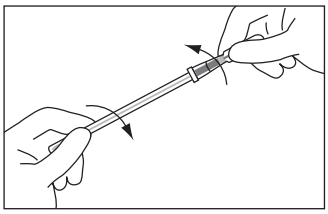


3 Squeeze the bulb of the swab to transfer all of the letheen broth to the tube end of the swab.

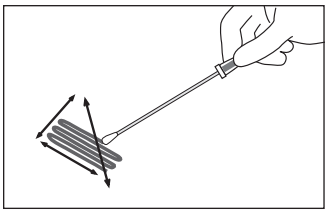
Alternative Swab Method

3M Petrifilm Plates can be used with other swabbing techniques, however the rinse solution used must be compatible with 3M Petrifilm Plates.

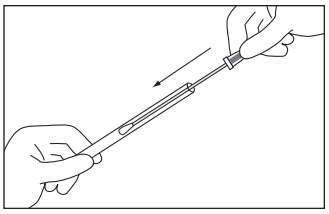
*For 3M Quick Swab dry swabbing method, see 3M Quick Swab product instructions.



4 Twist and pull apart the bulb end of the swab from the tube end of the swab which contains the letheen broth.



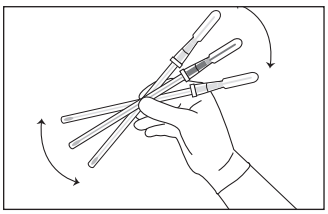
5 Hold the swab handle to make a 30° angle with the surface. Firmly rub the swab head slowly and thoroughly over the desired surface area. Rub the head of the swab three times over the surface, reversing direction between alternating strokes.



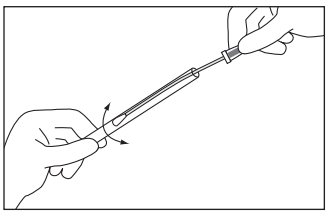
6 After sampling is complete, securely insert the swab head back into the swab tube and transport to the lab for plating. Plate the letheen broth swab solution as soon as possible.

Inoculation Procedures

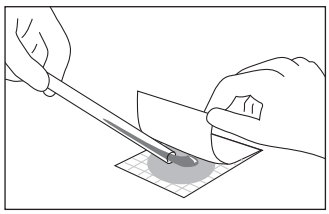
1 mL Inoculation Procedure



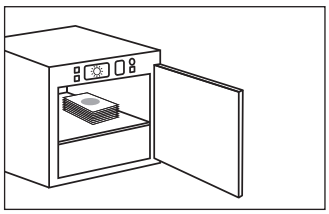
7a In the lab, vigorously shake or vortex the swab for 10 seconds, to release bacteria from the swab tip.



8a Release the contents of the swab tip by pressing and twisting the swab against the wall of the tube.

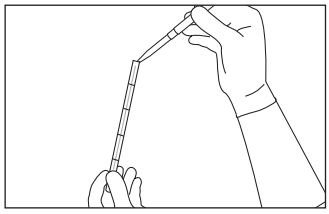


9a Carefully pour entire contents of the tube onto a 1 mL 3M Petrifilm Plate. Follow current industry standards for disposal.

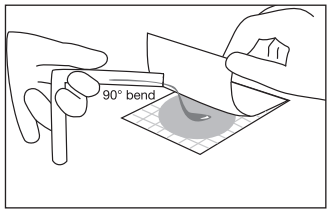


10a Incubate and enumerate as directed in product instructions. Refer to 3M Petrifilm Plate Interpretation Guide when enumerating results.

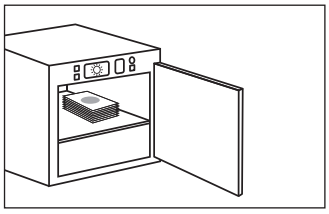
Multi-mL Inoculation Procedure



7b Remove the swab from the tube. Add 1–3 mL of sterile diluent to the swab tube. Replace the swab in the tube. Complete steps 7a and 8a of the 1 mL Inoculation Procedure from above.



8b Use your thumb to bend the swab tube at a 90° angle at the highest mark that has diluent above it. Pour off a 1 mL aliquot onto a 3M Petrifilm Plate. Repeat onto a new plate until the entire sample is used.



9b Incubate and enumerate as directed in product instructions. Refer to 3M Petrifilm Plate Interpretation Guide when reading results.

3M Quick Swab Method Results

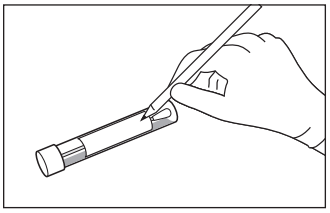
1 mL Inoculation Procedures:
3M Petrifilm Plate count x volume of diluent (1 mL) = total count/area sampled.

Example: If area tested was 5cm² and number of colonies on plate after incubation was 100, your result would be: 100 CFU x 1 mL = 100 CFU/5 cm²

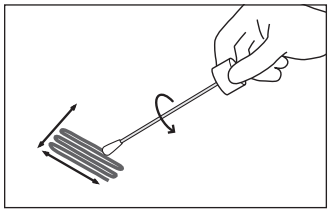
Multi-mL Inoculation Procedures: 3M Petrifilm Plate count x volume of diluent (1 mL + added) = total count/area sampled.

Example: If area tested was 5cm² and 2 mL were added (for total of 3 mL) and number of colonies after incubation was 100, your result would be: 100 CFU x 3 mL = 300 CFU/5 cm²

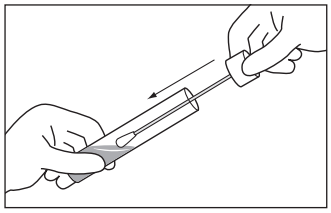
3M™ Swab Sampler Method



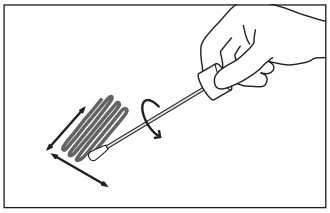
1 Label the 3M Swab Sampler. Unscrew the cap from the tube and aseptically remove the swab from the tube.



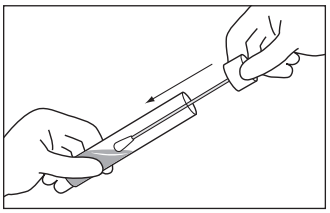
2 Aseptically swab across the sampling surface while rotating the swab.



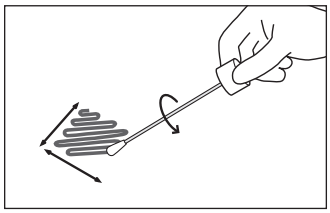
3 Return swab to the tube.



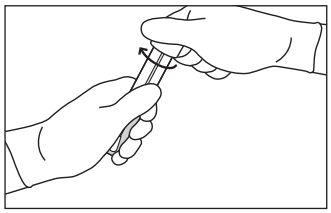
4 Repeat Step 2. Change direction 90° and aseptically swab the surface while rotating the swab.



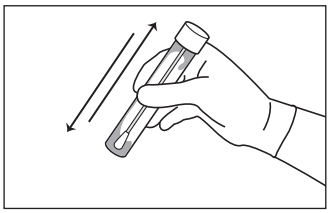
5 Return swab to the tube.



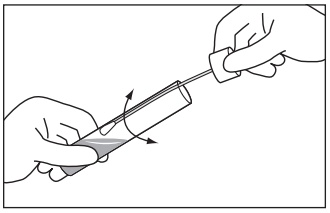
6 Repeat Step 2. Change direction 45° and aseptically swab the same sampling surface while rotating the swab.



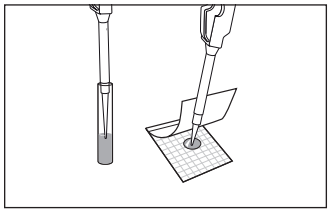
7 Return swab to the tube. Screw cap tight to close.



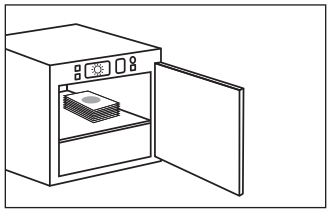
8 In the lab, vigorously shake or vortex the swab for 10 seconds, to release bacteria from the swab tip.



19 Unscrew the cap, release out the contents of the swab tip by pressing and twisting the swab against the wall of the tube. Remove swab from tube.



10 Using a pipettor with a sterile tip, draw 1 mL from the tube and dispense onto a 3M Petrifilm Plate. Repeat for additional plates as needed.



11 Incubate and enumerate as directed in product instructions. Refer to 3M Petrifilm Plate Interpretation Guide when reading results.

3M Swab Sampler Results

3M Petrifilm Plate count x volume of 3M Swab Sampler = total count/area sampled.

Example: If area tested was 5 cm² and a 4 mL 3M Swab Sampler was used and number of colonies on plate after incubation was 100, your result would be:
100 CFU x 4 mL = 400 CFU/5 cm²

Letheen broth 3M Swab Samplers are available in variety of sizes: 1 mL, 4 mL, 5 mL, 10 mL