NEOGEN®

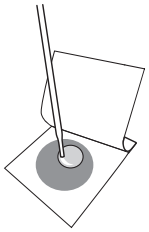
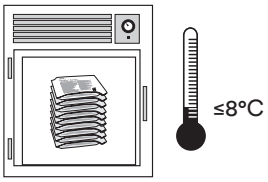
Petrifilm®

Environmental Monitoring Procedure

Neogen® Petrifilm® Plates

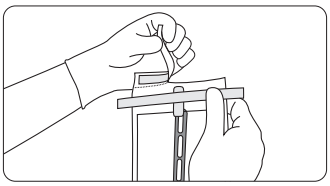
Petrifilm Plates are a convenient and reliable way to detect environmental microbial contamination. The construction of Petrifilm Plates allows them to be used for direct contact or swab contact monitoring procedures, as well as air sampling procedures.

Hydration Procedures for Air or Direct Contact Methods

Petrifilm Plate	Hydration*	Storage
AC Aerobic Count **CC** Coliform Count **RCC** Rapid Coliform Count **EC** *E. coli*/Coliform Count **REC** Rapid *E. coli*/Coliform Count **EB** *Enterobacteriaceae* Count **YM** Yeast and Mold Count **RYM** Rapid Yeast and Mold Count	 **Hydrate** plates with 1 mL of appropriate sterile diluent and allow hydrated plates to remain closed for a minimum of 1 hour before use.	 **Store** all hydrated Petrifilm Plates in sealed pouch or plastic bag. Protect plates from light and refrigerate at 2–8°C (36–46°F). Hydrated Petrifilm Aerobic Count Plates may be refrigerated for up to 14 days. Hydrated Petrifilm Rapid Yeast and Mold Count Plates may be refrigerated for up to 1 day (24 hours). All other hydrated Petrifilm Plates may be refrigerated for up to 7 days.
STX 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.	
RAC 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.	

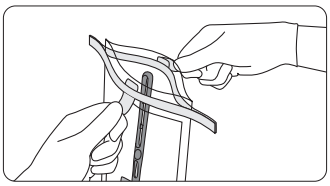
*See relevant Petrifilm Plate product instructions for details and listing of appropriate diluents. If sanitizers are present, use letheen broth for both the direct contact and swab contact methods.

Environmental Scrub Sampler with Wide Spectrum Neutralizer Method



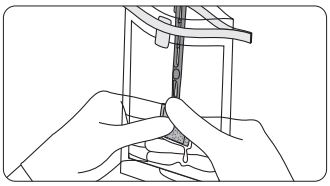
01

Wearing gloves, tear off the top of the bag along the perforation.



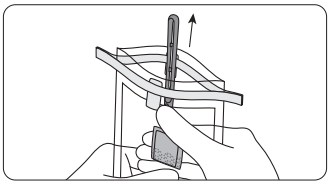
02

Aseptically open the bag using the red tabs on either side of the bag. Be sure not to touch the inside or edges of the bag.



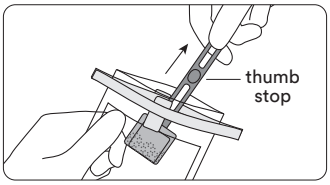
03

Squeeze out excess Neutralizer solution so the Neogen Environmental Scrub Sampler device is moist but not dripping.



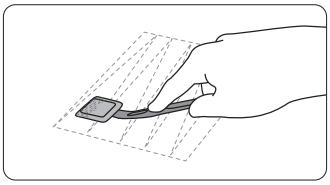
04

Working from the outside of the bag, move the device up allowing the stick to protrude from the bag.



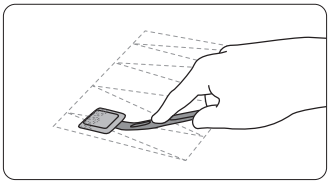
05

Aseptically, using one hand, grasp the stick above the thumb stop and remove the device from the bag, being sure the device does not touch the outside part of the bag.



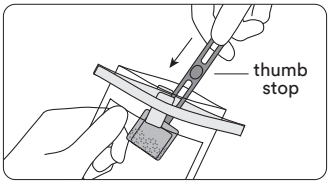
06

Practicing aseptic technique, press the Environmental Scrub Sampler device down firmly and flex the stick to ensure full contact with the sampling surface. Sample in a zigzag motion in one direction across the entire sampling surface. Optionally, scour vigorously in a zigzag motion in one direction across the entire sampling surface to disrupt organic matter if present. Sample an area from 10 cm x 10 cm (4 in x 4 in) to 30 cm x 30 cm (12 in x 12 in), following appropriate standards or regulatory guidance.[1,2,3,4,5]



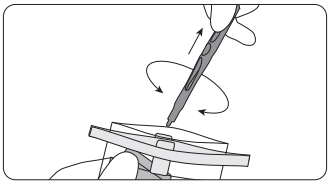
07

Turn the device over to the other side, change sampling direction by 90° and repeat the swabbing procedure described in step 06 in the same sampling site.



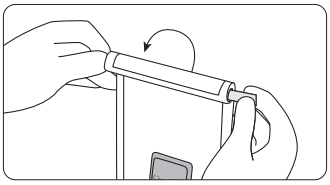
08

Return the sampling device back into the bag, without going beyond the thumb stop, and hold the device with one hand from the outside of the bag.



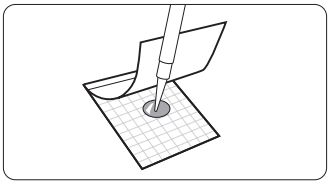
09

Using the other hand hold the Environmental Scrub Sampler Stick and twist it to separate from the Neogen Environmental Scrub Sampler device. Allow the Neogen Environmental Scrub Sampler to drop to the bottom of the bag to re-submerge into the Neutralizer. Discard the stick.



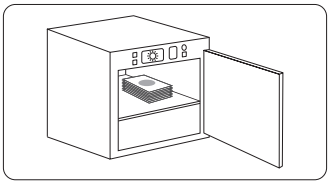
10

Close the bag by rolling the blue wires down and folding in the ends of the wires.



11

Using a pipettor with a sterile tip, draw 1 mL of the sample and dispense onto a Petrifilm Plate. Repeat for additional plates as needed.



12

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

Environmental Scrub Sampler Method Results

Petrifilm Plate count x volume of Environmental Scrub Sampler = total count/area sampled.

Example: If area tested was 30 cm^2 and the Environmental Scrub Sampler containing 10 mL of Wide-Spectrum Neutralizer was used and the number of colonies on plate after incubation was 30, the result would be: 30 CFU x 10 mL = 300 CFU/30 cm^2

The Neogen Wide-spectrum neutralizer is compatible with the following Petrifilm Plates

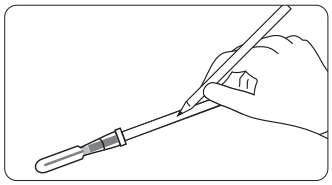
AC	RAC	EB
CC	HSCC	LAB
EC	REC	SEC
YM	RYM	

Please note that STX and RCC are not compatible with this method.

1. ISO 18593:2018. Microbiology of the food chain – Horizontal methods for surface sampling.
2. American Public Health Association — Compendium of Methods for the Microbiological Examination of Foods — Chapter 3: Microbiological Monitoring of the Food Processing Environment, 4th edition.
3. US Food and Drug Administration — Bacteriological Analytical Method (available online at https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam).
4. United States Department of Agriculture — Microbiological Lab Guidebook — Chapters 4.04, 5.04, and 8.07.
5. American Public Health Association — Standard Methods for the Examination of Dairy Products — Chapter 13: Microbiological Tests for Equipment, Containers, Water, and Air, 17th edition.

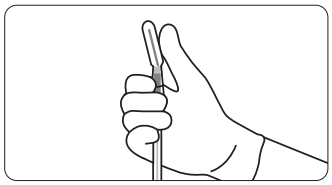
It is recommended that the surface area sampled should be cleaned after sampling using accepted cleaning procedures.

Quick Swab Method (wet swab method)*



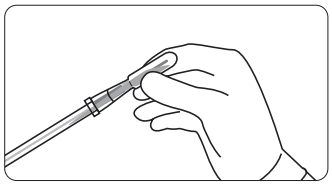
01

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



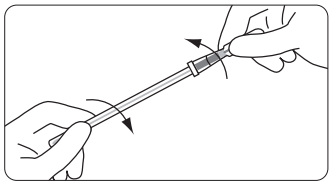
02

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.



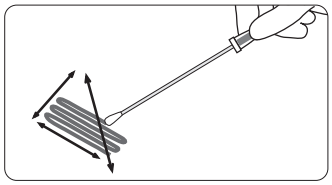
03

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



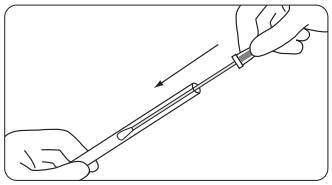
04

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



05

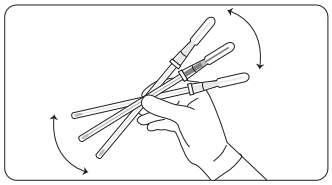
Hold the swab handle to make a 30° angle with the surface. Firmly rub the swab head slowly and thoroughly over the desired surface area. Rub the head of the swab three times over the surface, reversing direction between alternating strokes.



06

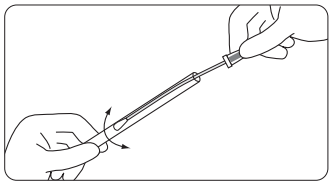
After sampling is complete, securely insert the swab head back into the swab tube and transport to the lab for plating. Plate the letheen broth swab solution as soon as possible.

1 mL Inoculation



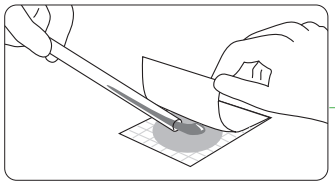
07a

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



08a

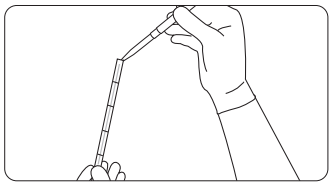
Release the contents of the swab tip by pressing and twisting the swab against the wall of the tube.



09a

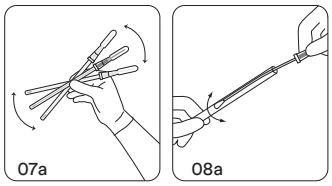
Release the contents of the swab tip by pressing and twisting the swab against the wall of the tube.

Multi-mL Inoculation



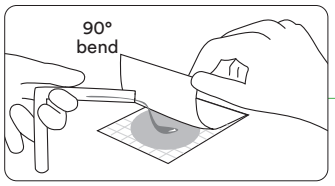
07b

Remove the swab from the tube. Add 1–3 mL of sterile diluent to the swab tube. Replace the swab in the tube.



08b

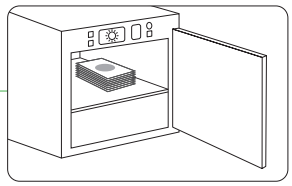
Complete steps 07a and 08a of the 1 mL Inoculation procedure from above.



09b

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 Petrifilm Plate. Repeat onto a new plate until the entire sample is used.

Incubation



10

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

Quick Swab Method Results

1 mL Inoculation:

Petrifilm Plate count x volume of diluent (1 mL) = total count/area sampled.

Example: If area tested was 10 cm^2 and number of colonies on plate after incubation was 100, the result would be: 100 CFU x 1 mL = 100 CFU/10 cm^2

Multi-mL Inoculation:

Petrifilm Plate count x volume of diluent (1 mL + added) = total count/area sampled.

Example: If area tested was 10 cm^2 and 2 mL were added (for total of 3 mL) and number of colonies after incubation was 100, the result would be: 100 CFU x 3 mL = 300 CFU/10 cm^2

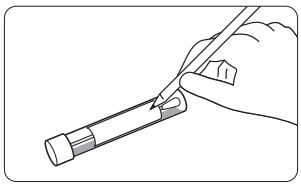
Alternative Swab Method

Petrifilm Plates can be used with other swabbing techniques, however the buffer solution or collection media used must be compatible with the Petrifilm Plates.

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

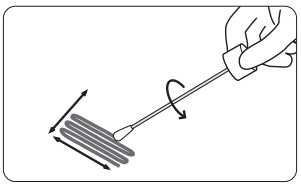
It is recommended that the surface area sampled should be cleaned after sampling using accepted cleaning procedures.

Swab Sampler Method



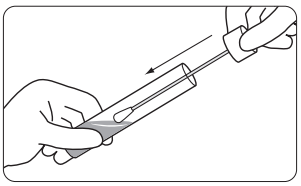
01

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



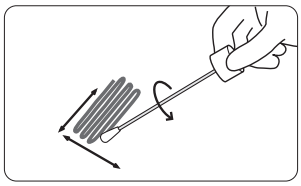
02

Aseptically swab across the sampling surface while rotating the swab.



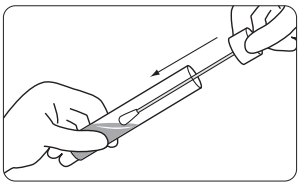
03

Return swab to the tube.



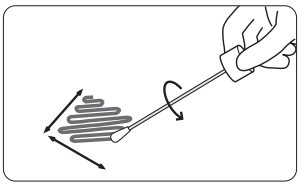
04

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



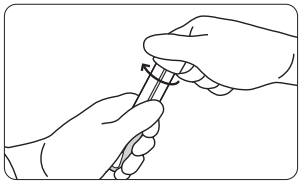
05

Return swab to the tube.



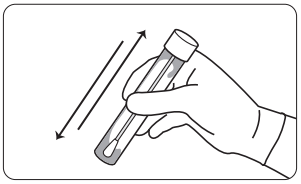
06

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



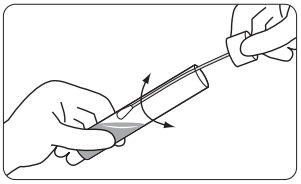
07

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



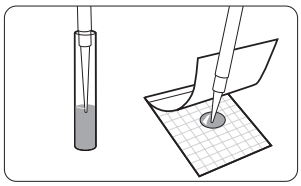
08

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



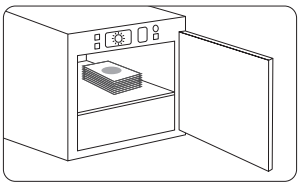
09

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 Petrifilm Plate. Repeat for additional plates as needed.



11

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

Swab Sampler Method Results

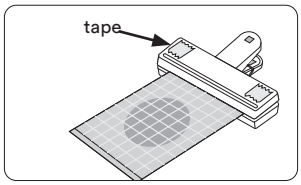
Petrifilm Plate count x volume of Swab Sampler = total count/area sampled.

Example: If area tested was 10 cm^2 and a 4 mL Swab Sampler was used and the number of colonies on plate after incubation was 100, the result would be: 100 CFU x 4 mL = 400 CFU/10 cm^2

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

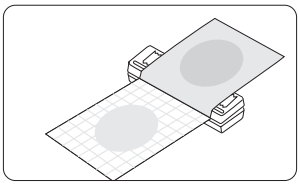
It is recommended that the surface area sampled should be cleaned after sampling using accepted cleaning procedures.

Air Sampling Method



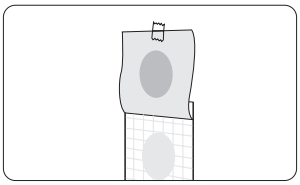
01

Use a Petrifilm Plate clip in combination with double-sided tape. Position hinged edge of hydrated 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 Petrifilm Plates for air sampling.



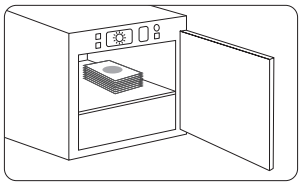
02

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.



03

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



04

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

Air Sampling Method Results

Petrifilm Plates

AC | CC | RCC | EB | EC

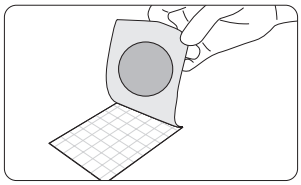
Results: count/40 cm^2

Petrifilm Plates

STX | YM | RYM | RAC | REC

Results: count/60 cm^2

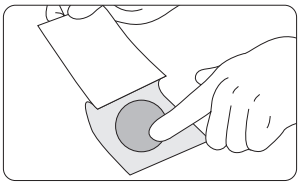
Direct Contact Method



01

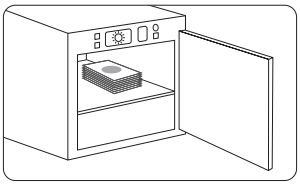
Using a hydrated Petrifilm Plate, carefully lift top film. Avoid touching circular growth area. Gel will adhere to top film.

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.



02

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.



03

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

Direct Contact Method Results

Petrifilm Plates

AC | CC | RCC | EB | EC

Results: count/20 cm^2

Petrifilm Plates

STX | YM | RYM | RAC | REC

Results: count/30 cm^2

It is recommended that the surface area sampled should be cleaned after sampling using accepted cleaning procedures.

Neogen offers a full line of products to accomplish a variety of your microbial testing needs.

For more product information, visit **info.neogen.com/Petrifilm**

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