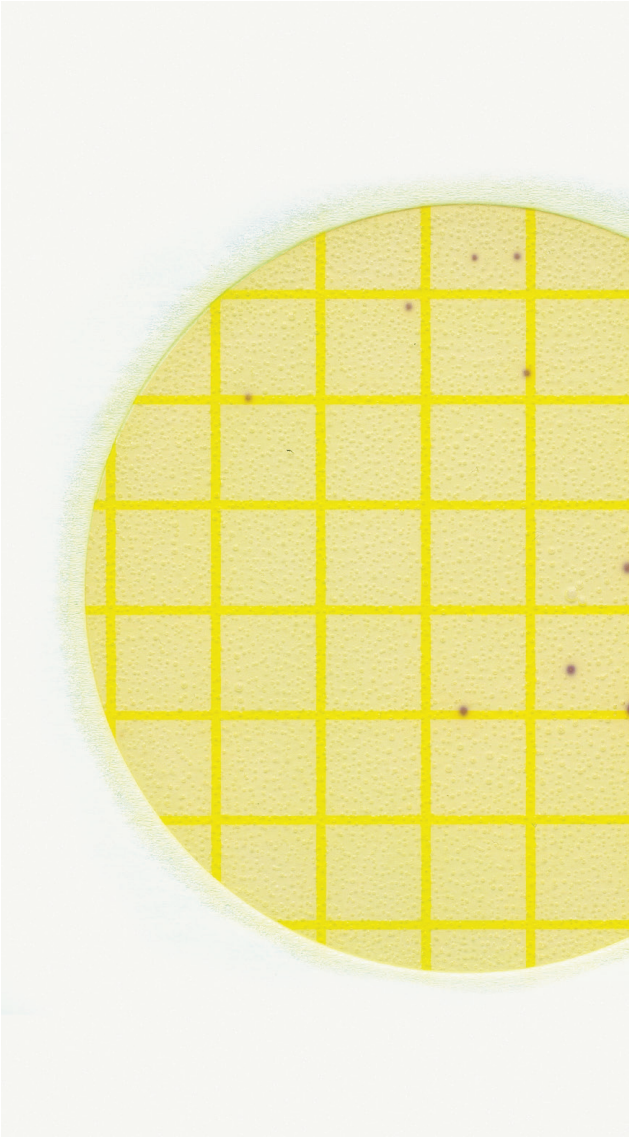
NEOGEN®

Petrifilm®

Interpretation Guide

The Neogen® Petrifilm® Environmental *Listeria* Plate is a sample-ready culture medium containing selective agents, nutrients, a cold-water-soluble gelling agent, and a chromogenic indicator that facilitates *Listeria* colony detection. Petrifilm Environmental *Listeria* Plates were designed to analyze environmental samples.



EL

Environmental *Listeria* Plate

The presence of indicator *Listeria* such as *Listeria innocua* provides evidence that environmental conditions are suitable for the occurrence of *Listeria monocytogenes*. The Petrifilm Environmental *Listeria* Plate detects the majority of environmental *Listeria*, consisting of *Listeria monocytogenes*, *Listeria innocua*, and *Listeria welshimeri*. *L. ivanovii, L. grayi/murrayi and L. seeligeri* grow but do not form typical colonies.

Many organisms in the environment can be stressed by environmental conditions or sanitizers. Buffered peptone water (BPW) is used as a repair broth in conjunction with the Petrifilm Environmental *Listeria* Plate to resuscitate stressed *Listeria* without increasing their numbers. Repair in BPW is not an enrichment step.

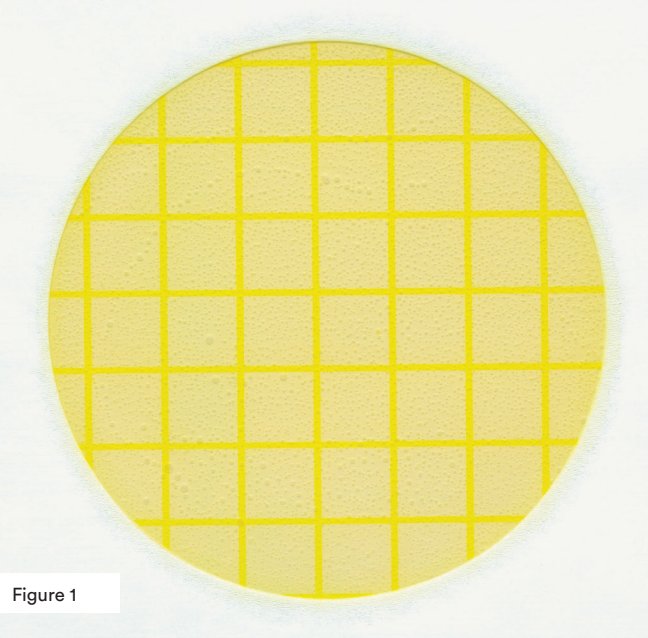


Figure 1

This Petrifilm Environmental *Listeria* Plate has no colonies after 28 hours of incubation. The test is complete.

- **Quantitative Interpretation:** *Listeria* colonies on this plate: 0. Please refer to the "Quantitative Sampling" section of this guide for calculating the quantity of *Listeria* per environmental sample.
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation:** *Listeria* not detected.

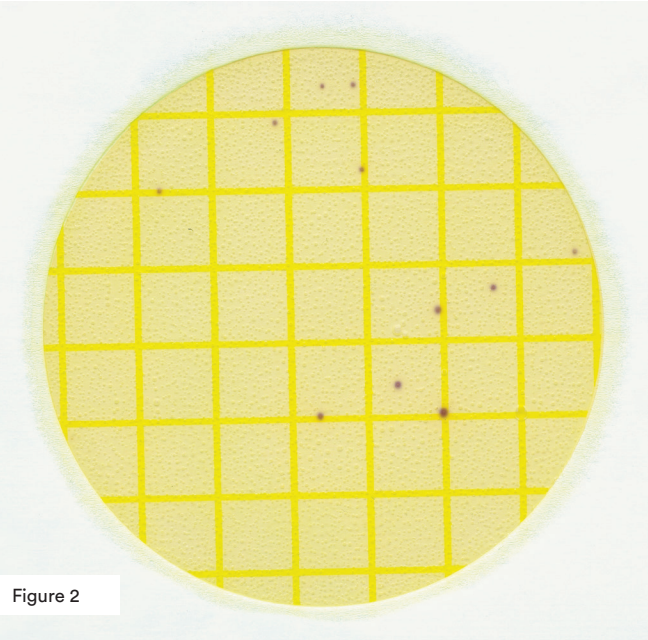


Figure 2

This Petrifilm Environmental *Listeria* Plate has only intense red-violet colonies after 28 hours of incubation. The test is complete.

- **Quantitative Interpretation:** *Listeria* colonies on this plate: 11.
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation**: *Listeria* detected.

Several factors influence the rate at which the chromogenic indicator changes to intense red-violet, including the strain and the nature and degree of stress to which the organism has been exposed.

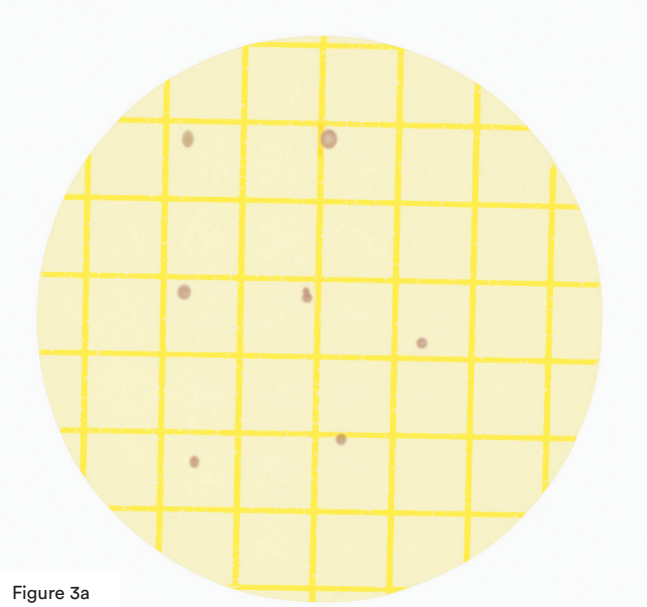


Figure 3a

Prior to the full 30 hour incubation, if any colonies are present but **are not** intense red-violet (for example, grey or light pink, as shown in 3a), then continue incubating up to 30 hours. At the maximum incubation time of 30 hours, colonies that do not turn intense red-violet (colonies **remain** grey or light pink, as shown in 3a), should not be interpreted as *Listeria*.

- **Quantitative Interpretation:** *Listeria* colonies on this plate: 0.
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation:** *Listeria* not detected.

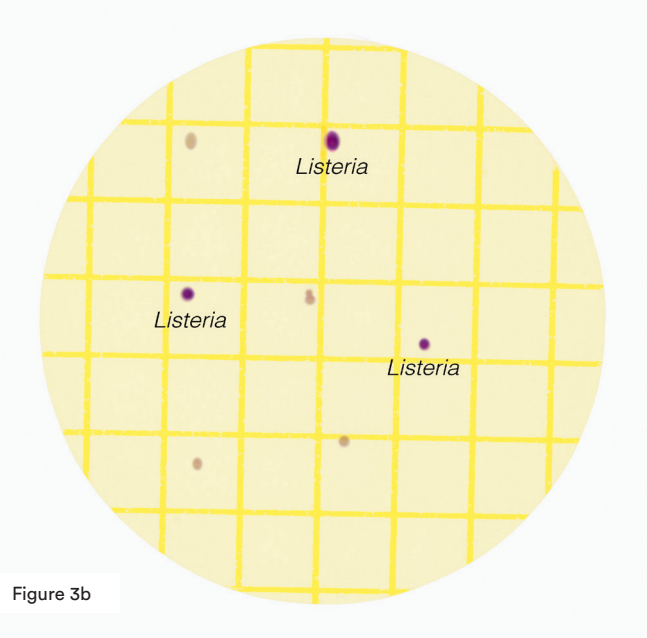


Figure 3b

At the maximum incubation time of 30 hours, colonies that were grey or light pink and **changed** to intense red-violet during incubation (as shown in 3b) should be interpreted as *Listeria*.

- **Quantitative Interpretation:** *Listeria* colonies on this plate: 3.
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation:** *Listeria* detected.

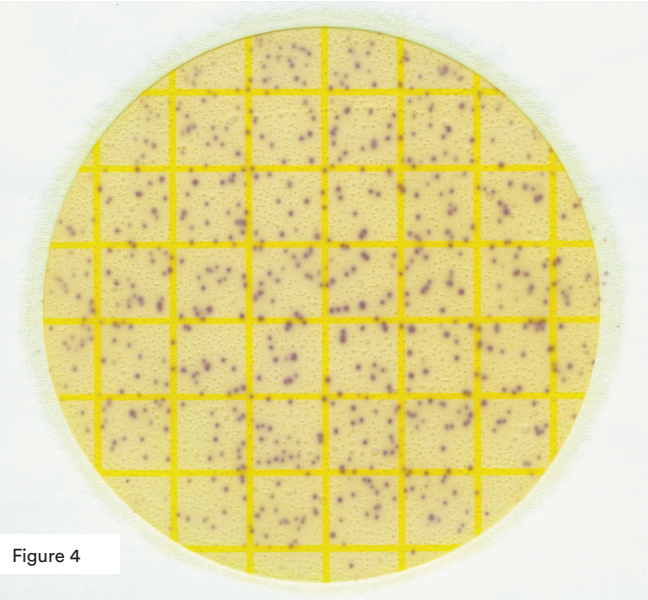


Figure 4

Since the Petrifilm Environmental *Listeria* Plate may be interpreted in three ways, no counting range is suggested. When colonies are crowded, interpret the result (qualitative or semi-quantitative) or estimate the count (quantitative) as described below.

- **Quantitative Interpretation:** Estimated *Listeria* colonies on this plate: 600. When large numbers of *Listeria* are present, estimate by determining the count per square of two or more representative squares. Determine the average per square and then multiply by 42. The inoculated area of the plate is approximately 42 cm^2.
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation:** *Listeria* detected.

Note: Do not consider or count colonies on the foam dam since they are removed from the selective influence of the medium.

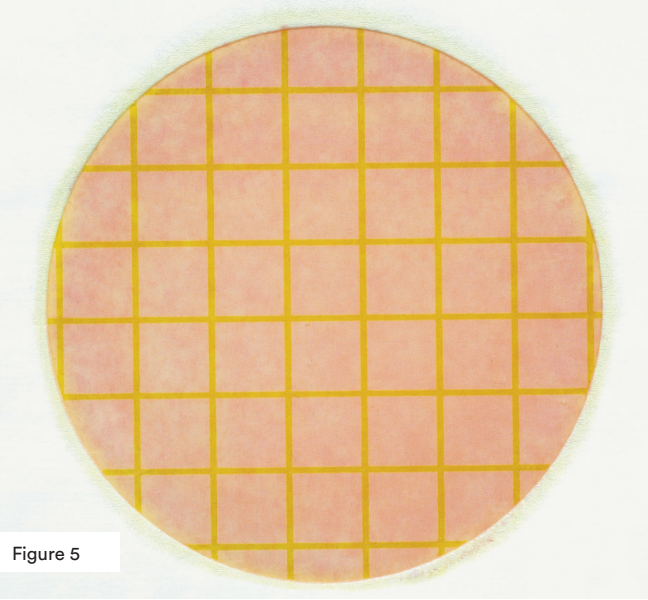


Figure 5

When colonies are present in large numbers, the Petrifilm Environmental *Listeria* Plate may have many small, indistinct colonies and/or a pink-brown color throughout.

- **Quantitative Interpretation:** *Listeria* colonies on this plate are too numerous to count (TNTC).
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation:** *Listeria* detected.

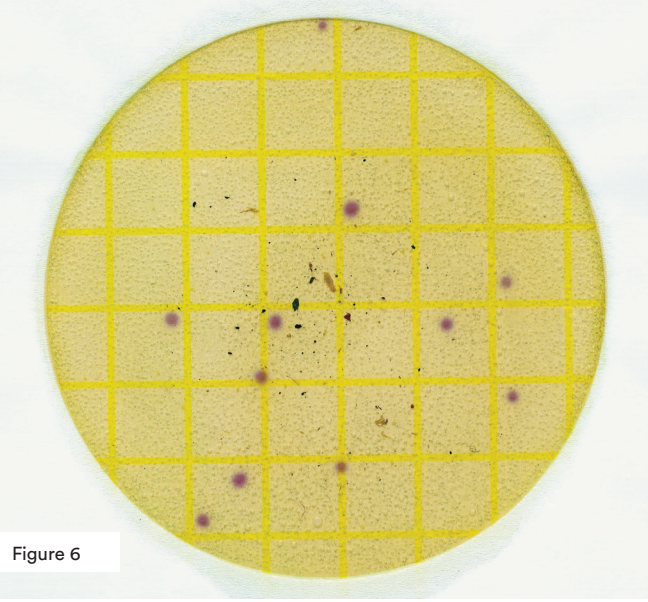


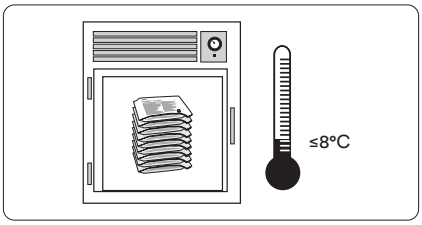
Figure 6

Background color may vary due to the presence of dust, soil, grit, or other sediment from the environment sampled, or the type of sample collection device and/or the brand of buffered peptone water (repair broth). Interpret or count the intense red-violet colonies as *Listeria*.

- **Quantitative Interpretation:** *Listeria* colonies on this plate: 11.
- **Semi-Quantitative Interpretation:** *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).
- **Qualitative Interpretation:** *Listeria* detected.

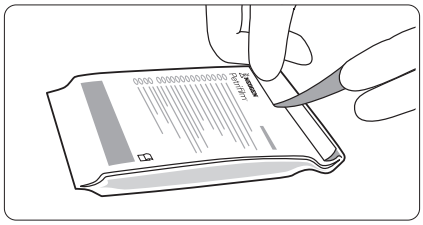
Reminders For Use

Storage



01

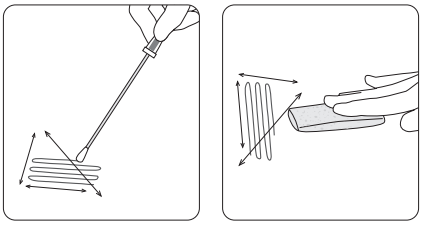
Store unopened pouches at ≤8°C (≤46°F). Use before expiration date on package. Just prior to use, allow the unopened pouches to come to room temperature before opening.



02

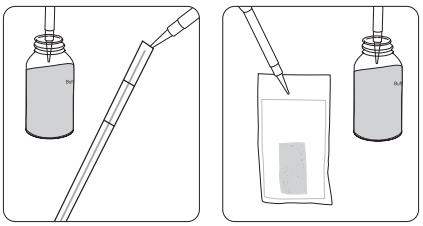
To seal opened pouch, fold end of the pouch over and apply adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than four weeks.

Sample Preparation



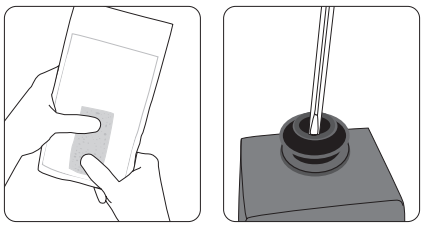
03

Collect environmental samples using a Quick Swab or equivalent, sponge or other moistened collection device. The moistening agent may be ≤10 mL sterile water, buffered peptone water (BPW) or if sanitizers are present, neutralizing buffer such as letheen broth or neutralizing broth is recommended.



04

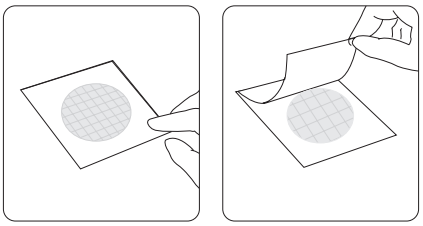
Aseptically add 2 mL (swab) or 5 mL (sponge) sterile buffered peptone water (20°C–30°C) to the collected sample. Do not use enrichment broth on this plate.



05

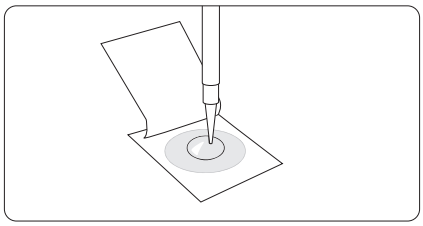
Vigorously mix, stomach or vortex the collected sample with BPW for approximately one minute. Allow the sample to remain at room temperature, 20°C–30°C, for 1 hour up to a maximum of 1.5 hours, then vigorously mix again. This step is required for repair of injured *Listeria*. For optimal bacterial growth or recovery the sample should have a pH between 4 and 9.

Inoculation



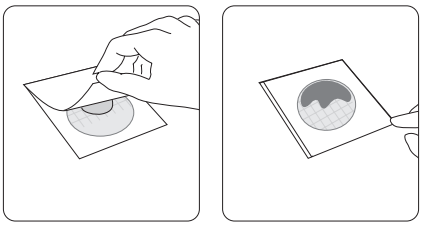
06

Place Petrifilm Environmental *Listeria* Plate on level surface. Lift top film.



07

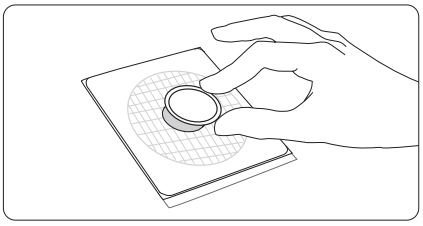
Lift the top film and, with the pipette perpendicular to the inoculation area, dispense 3 mL of sample suspension onto the center of the bottom film.



08

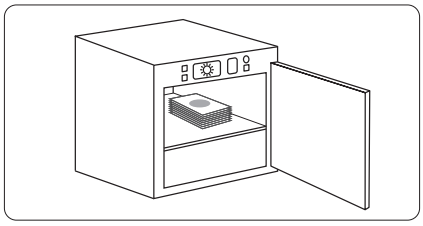
Roll the top film down onto the sample.

Incubation



09

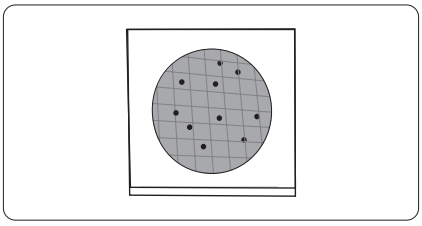
Gently place the Petrifilm Large Square Spreader on the top film over the inoculum. **Do not** press, twist or slide the spreader. Lift spreader. Wait at least 10 minutes to permit the gel to form.



10

Incubate plates with clear side up in stacks of up to 10 for 28 h ± 2h at 35°C ± 1°C or 37°C ± 1°C. Do not exceed 30 hours. Incubation beyond the recommended time may yield ambiguous results. **Please refer to product instructions for third party validated methods.**

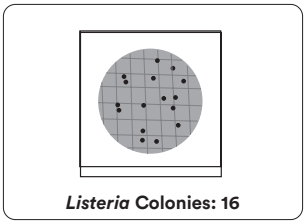
Interpretation



11

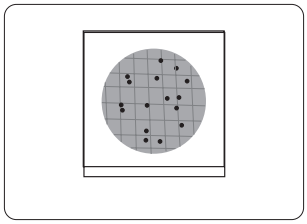
Petrifilm Environmental *Listeria* Plates can be counted or interpreted using a standard colony counter or other illuminated magnifier. Do not count colonies on the foam dam since they are removed from the selective influence of the medium.

The Petrifilm Environmental *Listeria* Plate method can be used as a quantitative, semi-quantitative or qualitative test.



12

For a **quantitative** test, count and record all intense red-violet colonies. You may wish to choose a quantitative test if you take different actions based upon the number present. Please refer to the "Quantitative Sampling" section of this guide for calculating the quantity of *Listeria* per environmental sample.

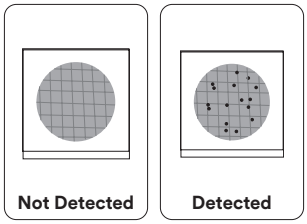


13

For a **semi-quantitative** test, record results based on the **relative number** of intense red-violet colonies present.

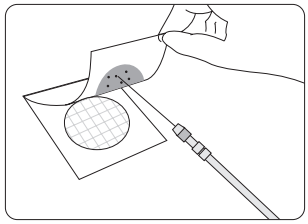
You may wish to choose a semi-quantitative test if you take different actions depending on the relative level present, and if recording an actual number is not required.

Listeria level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).



14

For a **qualitative** test, record results of the plate as **detected** or **not detected** based on the presence or absence of intense red-violet colonies. You may wish to choose a qualitative test if a yes/no answer is sufficient and appropriate for your reporting.



15

Optional: Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Quantitative Sampling & Interpretation

If your facility chooses to use the Petrifilm Environmental *Listeria* Plate in a quantitative manner, please refer to the product instructions, and then calculate the colony forming units (CFU) per area as shown below. You may also want to consider the following points:

- Consistency is the key to obtaining useful information from your environmental monitoring program. Use a consistent procedure each time that you sample. Ideally, use the same type of sampling device and techniques.
- The sampling area size may be based on regulations, internal standards, and/or the location of the monitoring.
- More information on environmental sampling can be found at the references listed below, and in the Petrifilm Plates Environmental Monitoring Procedures brochure.

To determine the quantity of *Listeria* per sampled area, you will need to record:

1. **Area size sampled**
2. **Volume of hydration fluid in the sampling device**
3. **Volume of the buffered peptone water added**
4. **Volume plated**
5. **Number of colonies counted**

Apply the following equation or worksheet to determine the CFU/area sampled. Examples are given on the following page. See Product Instructions & Reminders for Use for full details of the method.

You may also determine the result per sample, e.g., CFU/drain.

CFU/area = (Number of colonies x [mL hydration fluid + mL BPW] ÷ 3 mL) ÷ area sampled

OR

A. Total number of mL of BPW + hydration fluid:	_____	A
B. Number of mL plated:	3 mL	B
C. Divide line A by line B:	_____	C
D. Number of colonies counted: (if number of colonies is zero, insert "<1" into line "D")	_____	D
E. Multiply line C by line D:	_____	E
F. Area sampled:	_____	F
G. Divide line E by line F:	_____	G

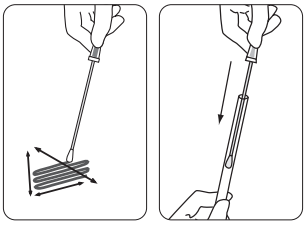
Line G equals CFU/area

Environmental quantitative sampling is consistent with the following references:

- Standard Methods for the Examination of Dairy Products, Section 3.084, American Public Health Association, Washington D.C. 2004, 17th edition.
- Compendium of Methods for the Microbiological Examination of Foods, Section 3.81 and 3.82, American Public Health Association, Washington D.C. 2015, 5th edition.

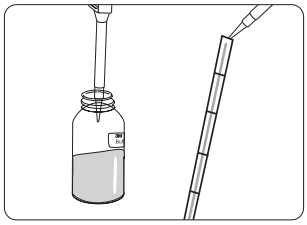
Quantitative Interpretation

Example: Quick Swab Contact Method



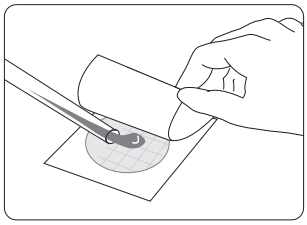
01

Using a Quick Swab (or equivalent) moistened with 1 mL of hydration fluid (see line A), sample an area. For this example, area is fifty square centimeters (50 cm^2) (see line F). Return Quick Swab to sterile container.



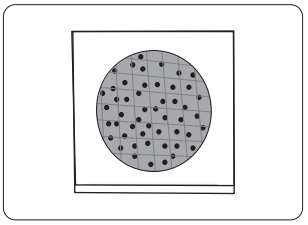
02

Add 2 mL of buffered peptone water (see line A).



03

After repair step, plate 3 mL onto the Petrifilm Environmental *Listeria* Plate (see line B).



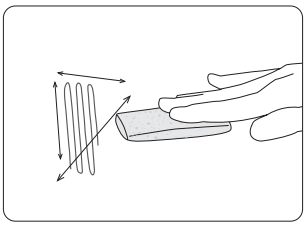
04

After incubation, count colonies. For this example, assume you count fifty (50) colonies (see line D).

A. Total number of mL of BPW + hydration fluid:	1+2=3	A
B. Number of mL plated:	3	B
C. Divide line A by line B:	1	C
D. Number of colonies counted:	50	D
E. Multiply line C by line D:	50	E
F. Area sampled:	50 cm^2	F
G. Divide line E by line F:	1 CFU/cm^2	G

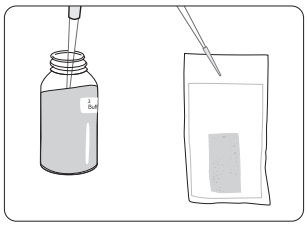
Line G equals CFU/area

Example: Sponge Contact Method



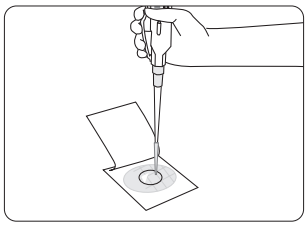
01

Using a sponge moistened with 10 mL of hydration fluid, sample an area (see line A). For this example, area is one square foot (1 ft^2) (see line F).



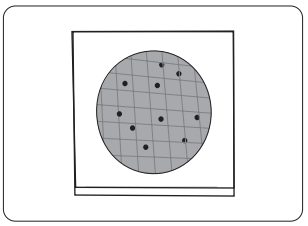
02

Return the sponge to the sterile container and add 5 mL of buffered peptone water (see line A).



03

After repair step, plate 3 mL onto the Petrifilm Environmental *Listeria* Plate (see line B).



04

After incubation, count colonies. For this example, assume you count ten (10) colonies (see line D).

A. Total number of mL of BPW + hydration fluid:	10+5=15	A
B. Number of mL plated:	3	B
C. Divide line A by line B:	5	C
D. Number of colonies counted:	10	D
E. Multiply line C by line D:	50	E
F. Area sampled:	1 ft^2	F
G. Divide line E by line F:	50 CFU/ft^2	G

Line G equals CFU/area

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**

User's Responsibilities: Neogen Petrifilm Plate performance has not been evaluated with all combinations of microbial flora, incubation conditions and food matrices. It is the user's responsibility to determine that any test methods and results meet the user's requirements. Should re-printing of this Interpretation Guide be necessary, user's print settings may impact picture and color quality.

For detailed CAUTIONS, DISCLAIMER OF WARRANTIES/LIMITED REMEDY and LIMITATION OF NEOGEN LIABILITY, STORAGE AND DISPOSAL information and INSTRUCTIONS FOR USE, see product instructions.

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