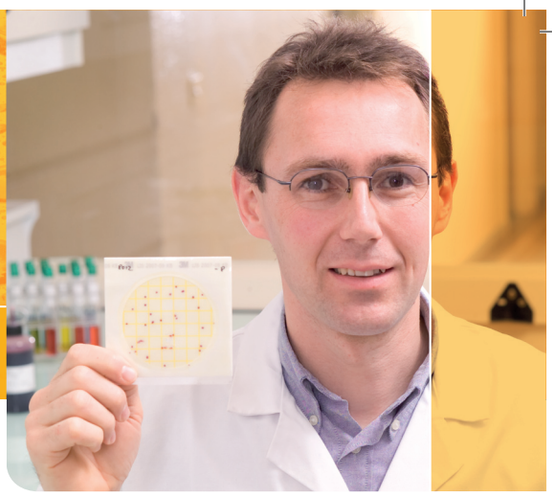


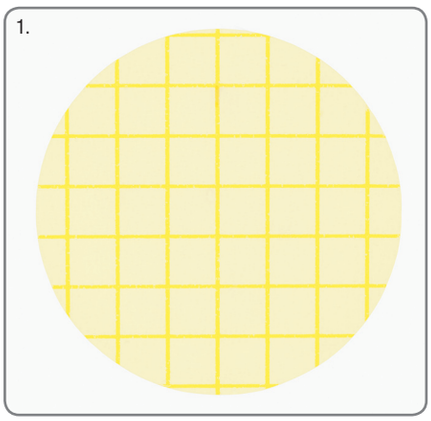
3M™ Petrifilm™ Interpretation Guide

3M™ Petrifilm™ Environmental Listeria Plates

This guide familiarizes you with results on 3M™ Petrifilm™ Environmental Listeria Plates. For more information, contact the 3M Microbiology representative nearest you.

The Petrifilm environmental Listeria (EL) 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 EL plates are designed to analyse environmental samples and to help increase the efficiency of monitoring plant sanitation. The presence of indicator *Listeria* such as *Listeria innocua* provides evidence that environmental conditions are suitable for the occurrence of *Listeria monocytogenes*. The Petrifilm EL plate detects the majority of environmental *Listeria*, consisting of *Listeria monocytogenes, Listeria innocua,* and *Listeria welshimeri.**

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 EL plate to resuscitate stressed *Listeria* without increasing their numbers. Repair in BPW is not an enrichment step.

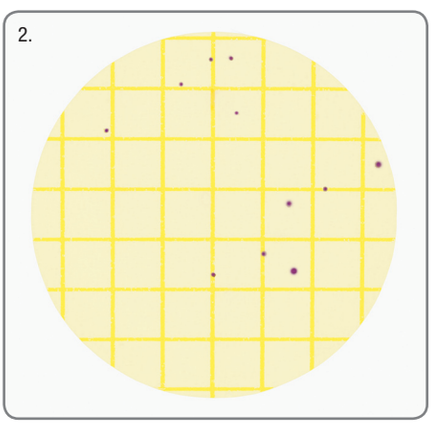


1. This Petrifilm EL plate has NO colonies after 28h of incubation.
   The test is complete.

   Quantitative Interpretation: *Listeria* colonies on this plate: **<1.** 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



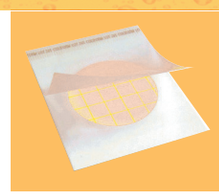
2. This Petrifilm EL plate has ONLY intense red-violet colonies after 28h of incubation.
   The test is complete.

   Quantitative Interpretation: *Listeria* colonies on this plate: **11.** 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).

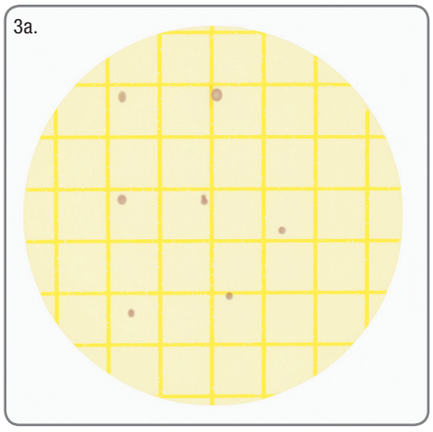
   Qualitative Interpretation: *Listeria* detected

* For further information on the prevalence of *Listeria* species, please contact the 3M Microbiology representative nearest you.
L. ivanovii, L. grayi/murrayi and *L. seeligeri* grow but do not form typical colonies.



3M™ Petrifilm™ Environmental Listeria Plate

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

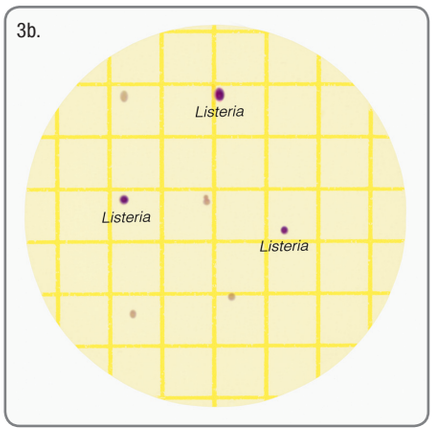


3a. Prior to the full 30 hour incubation, if any colonies are present but are not intense red-violet (for example, grey or light pink, as shown in 3a), then continue incubating up to 30 hours. At the maximum incubation time of 30 hours, colonies that do not turn intense red-violet (colonies remain grey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

Quantitative Interpretation: *Listeria* colonies on this plate: **<1.** 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.



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

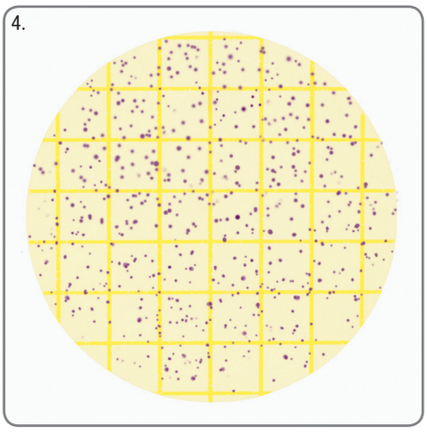
Quantitative Interpretation: *Listeria* colonies on this plate: **3.** Please refer to the "Quantitative Sampling" section of this guide for calculating the quantity of *Listeria* per environmental sample.

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* detected.

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

3M™ Petrifilm™ Environmental Listeria Plate

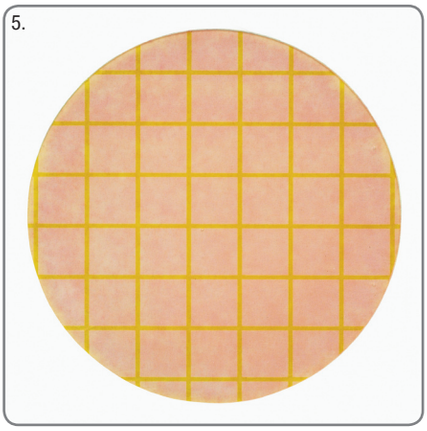


4. Since the Petrifilm EL plate may be interpreted in three ways, no counting range is suggested. When colonies are crowded, interpret the result (qualitative or semi-quantitative) or estimate the count (quantitative) as described below.

Quantitative Interpretation: Estimated *Listeria* colonies on this plate: **est. 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.

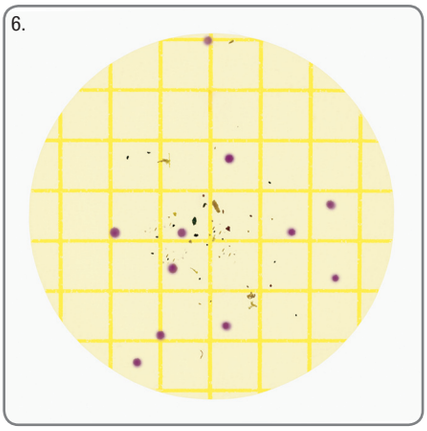


5. When colonies are present in large numbers, Petrifilm EL plates may have many small, indistinct colonies and/or a pink-brown colour throughout.

Quantitative Interpretation: *Listeria* on this plate is too numerous to count (TNTC, approximately 10^4 shown in this image).

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.



6. Background colour 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.** Please refer to the "Quantitative Sampling" section of this guide for calculating the quantity of *Listeria* per environmental sample.

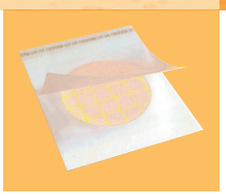
Semi-Quantitative Interpretation: *Listeria* level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).

Qualitative Interpretation: *Listeria* detected.

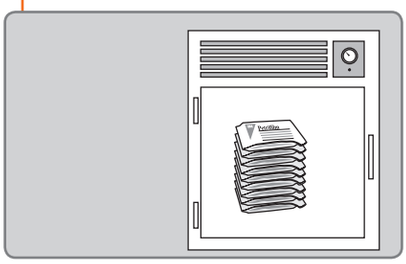
3M™ Petrifilm™ Environmental Listeria Plate

For detailed WARNINGS, CAUTIONS, DISCLAIMER OF WARRANTIES / LIMITED REMEDY, LIMITATION OF 3M LIABILITY, STORAGE AND DISPOSAL information, and INSTRUCTIONS FOR USE see Product's package insert.

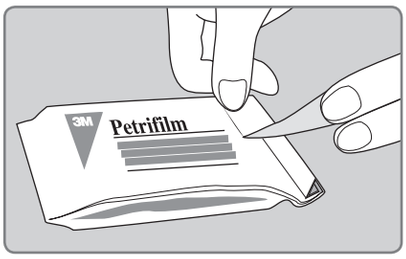
Reminders for Use



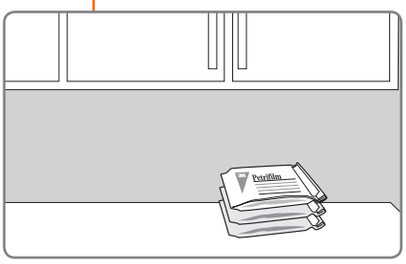
Storage



1 Store unopened pouches at ≤8°C (≤46°F). Use before expiration date on package. In areas of high humidity, it is best to allow pouches to reach room temperature before opening.

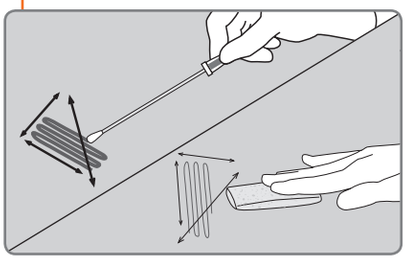


2 To seal opened pouch, fold end over and tape shut.



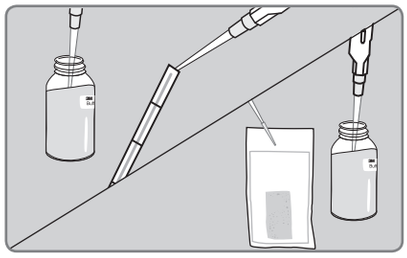
3 **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than one month. Avoid exposing plates to temperature >25°C (>77°F) and/or the relative humidity is >50%.

Sample Preparation



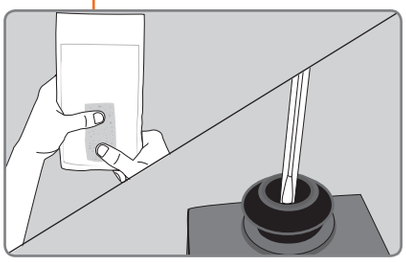
4 Collect environmental samples using a swab or equivalent, sponge or other moistened collection device.

The moistening agent should be ≤10 mL sterile water, buffered peptone water (BPW) or if sanitisers are present, neutralizing buffer such as Letheen Broth or Neutralising Broth is recommended.



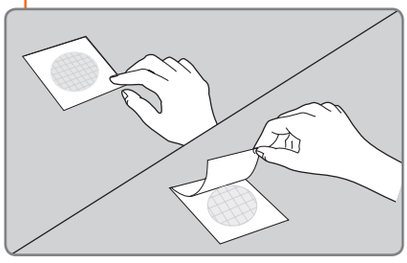
5 Aseptically add 2 mL (swab) or 5 mL (sponge) sterile 20°C–30°C (68°F – 86°F) buffered peptone water (repair broth) to the collected sample.

Do not use enrichment broth on this plate.

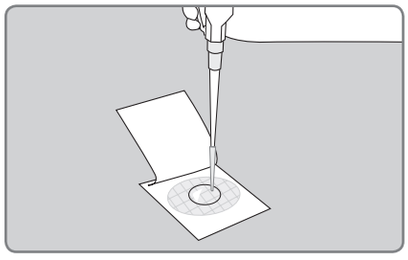


6 Vigorously mix, stomach or vortex the collected sample with BPW for approximately one minute. Allow the sample to remain at **room temperature, 20°C–30°C (68°F–86°F), for 1 hour up to a maximum of 1.5 hours, then vigorously mix again.** This step is required for repair of injured *Listeria*.

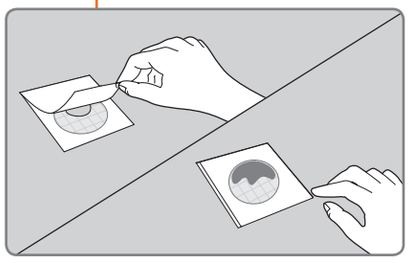
Inoculation



7 Place Petrifilm EL plate on level surface. Lift top film.

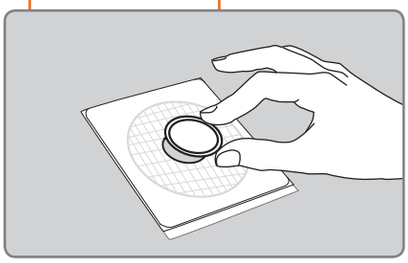


8 With 3M™ Electronic Pipettor or equivalent pipettor held perpendicular to Petrifilm EL plate, place **3 mL** of sample onto the center of bottom film.



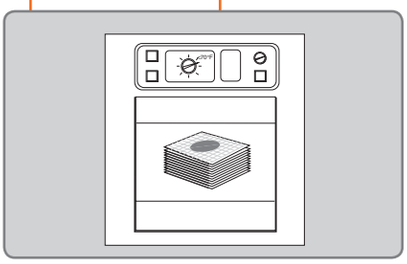
9 Roll the top film down onto the sample.

Inoculation



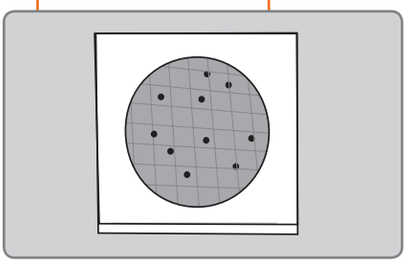
10 **Gently** place the plastic spreader on the top film over the inoculum. **Do not press**, twist or slide the spreader. Lift spreader. Wait at least **10 minutes** to permit the gel to form. Note: if the inoculum self-spreads, the spreader is not necessary.

Incubation



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

Interpretation

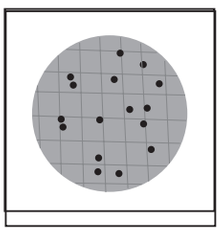


12 Petrifilm EL plates can be counted or interpreted using a standard colony counter or other illuminated magnifier. Do not count colonies on the foam dam since they are removed from the selective influence of the medium.

The 3M™ Petrifilm™ Environmental Listeria Plate method can be used as a quantitative, semi-quantitative or qualitative test.

13 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.

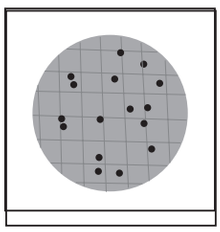


Listeria colonies on this plate: 16

Please refer to the „Quantitative Sampling“ section of this guide for calculating the quantity of *Listeria* per environmental sample.

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

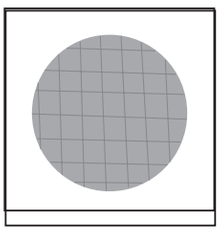
You may wish to choose a semi-quantitative test if you take different actions depending on the relative level present, and if recording an actual number is not required.



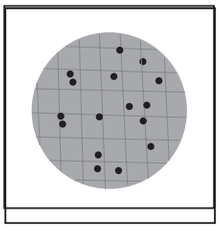
Listeria level should be recorded as categories that are meaningful to your sampling location and your individual plant standards (e.g., low, medium, high, or acceptable and unacceptable).

15 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.

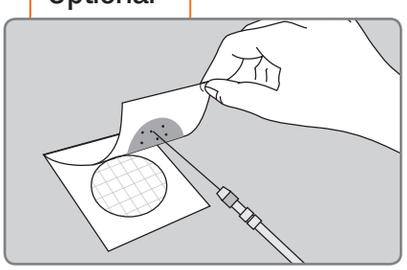


Not detected



Detected

Optional



16 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 package insert, 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 programme. 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. For example, you may need to sample a larger area for a finished goods area because the numbers of bacteria are expected to be low.
- More information on environmental sampling can be found in 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 pages.
See Package Insert & 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

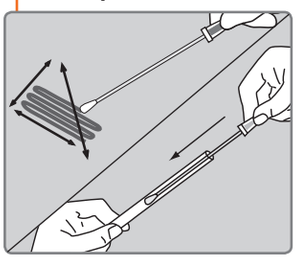
A. Total number of mL of BPW + hydration fluid:	_____	A
B. Number of mL plated:	3 mL	B
C. Divide line A by line B:	_____	C
D. Number of colonies counted: (if number of colonies is zero, insert “<1” into line “D”)	_____	D
E. Multiply line C by line D:	_____	E
F. Area sampled:	_____	F
G. Divide line E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

Environmental quantitative sampling is consistent with the following references:

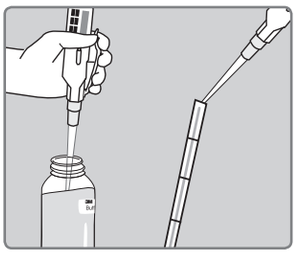
- Standard Methods for the Examination of Dairy Products, Section 3.7D, American Public Health Association, Washington D.C., 1992.
- Compendium of Methods for the Microbiological Examination of Foods, Section 3.512 and 3.521, American Public Health Association, Washington D.C., 2001.

Quantitative Interpretation

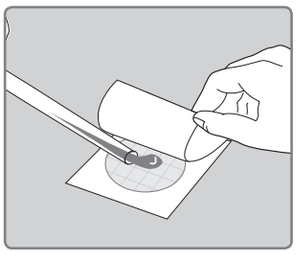
Example: Swab Contact Method



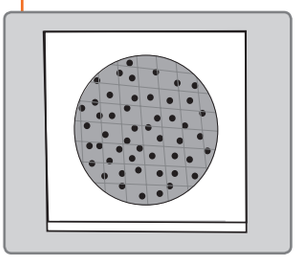
1 Using a swab (or equivalent) moistened with **1 mL** of hydration fluid (see line A), sample an area. For this example, area is fifty square centimeters (**50 cm²**) (see line F). Return swab to sterile container.



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



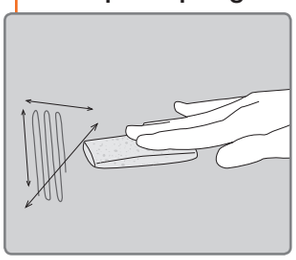
3 After repair step, plate **3 mL** onto the Petrifilm environmental Listeria plate (see line B).



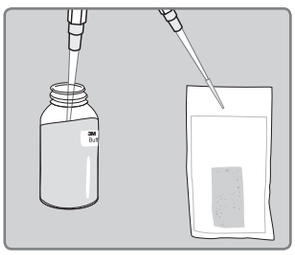
4 After incubation, count colonies. For this example, assume you count **fifty (50)** colonies (see line D).

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

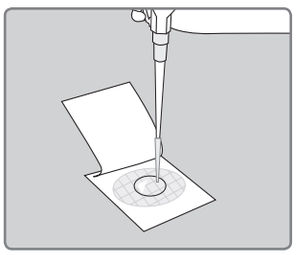
Example: Sponge Contact Method



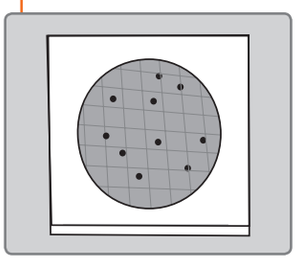
1 Using a sponge moistened with **10 mL** of hydration fluid, sample an area (see line A). For this example, area is fifty square centimeters (**50 cm²**) (see line F).



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

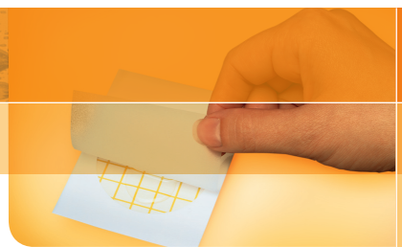


3 After repair step, plate **3 mL** onto the Petrifilm environmental Listeria plate (see line B).



4 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:	50 cm^2	F
G. Divide line E by line F:	1 CFU/cm^2	G



Additional Comments

3M Microbiology offers a full range of Petrifilm count plates designed to meet microbial testing requirements within the Food Industry.

For further product information please visit our website:

www.3M.com/microbiology

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