



Petrifilm™

6405/6415

## Product Instructions

High-Sensitivity Coliform Count Plate

**HSCC**

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## Product Instructions

# High-Sensitivity Coliform Count Plate

### Product Description and Intended Use

The 3M™ Petrifilm™ High-Sensitivity Coliform Count (HSCC) Plate is a sample-ready-culture medium system which contains modified Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm HSCC Plates are used for the enumeration of low levels of coliforms in the food and beverage industries. A 5 mL sample is plated onto one plate. This allows a 1-coliform-per-gram sensitivity if a 1:5 dilution of product made. A 2-coliform-per-gram sensitivity is achieved if a 1:10 dilution is used. 3M Petrifilm HSCC Plate components are decontaminated though not sterilized. 3M Food Safety is certified to International Organization for Standardization (ISO) 9001 for design and manufacturing. 3M Petrifilm HSCC Plates have not been evaluated with all possible food products, food processes, testing protocols or with all possible microorganism strains.

The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM)<sup>1</sup> define coliforms as Gram-negative rods, which produce acid and gas from lactose fermentation. Coliform colonies growing on the 3M Petrifilm HSCC Plate produce acid, which causes the pH indicator to deepen the gel color; gas trapped around red colonies indicates coliforms.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832<sup>4</sup>, enumerating coliforms by colony count technique, defines coliforms as acid producers on VRB with lactose (VRBL) agar. On 3M Petrifilm HSCC Plates these acid-producing coliforms are indicated by red colonies with or without gas production. ISO method 4831<sup>3</sup>, enumerating coliforms by the most probable number (MPN) method, defines coliforms by their ability to grow and produce gas from lactose in a selective broth. On 3M Petrifilm HSCC Plates these coliforms are indicated by red colonies associated with gas.

AFNOR has validated 3M Petrifilm HSCC Plate in comparison to ISO method 4831<sup>3</sup> for enumeration of total coliforms.

### Safety

The user should read, understand, and follow all safety information in the instructions for the 3M Petrifilm HSCC 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

### 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 instructions 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 HSCC 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<sup>6</sup>, ISO 17025<sup>7</sup> or ISO 7218<sup>8</sup>.

### To reduce the risks associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm HSCC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm HSCC Plates for testing water, pharmaceuticals or cosmetics.
- Do not use 3M Petrifilm HSCC Plates in the diagnosis of conditions in humans or animals.
- 3M Petrifilm HSCC Plates do not differentiate any one coliform strain from another.
- When testing certain unprocessed foods 3M Petrifilm HSCC Plates may produce a softer gel, which may or may not interfere with the ability to read the plate. Some of the foods that have been found to cause gel softening include some raw shellfish (including mussels, oysters, scallops and shrimp) and some raw flour (including buckwheat flour, graham flour and organic whole-wheat flour).
- High sugar content foods may increase growth potential for gas producing non-coliform *Enterobacteriaceae*.
- A high level of background organisms in certain food (for example, some cheeses and some chilled unprocessed foods including raw fish and raw milk) may elevate the number of red colonies **without** gas.

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 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' or 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 TO USER OR OTHERS FOR ANY LOSS OR DAMAGE, 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 HSCC 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 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 HSCC Plates be stored in a freezer 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 HSCC Plates in a sealable container. To remove frozen 3M Petrifilm HSCC 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 HSCC 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 3M Petrifilm HSCC Plates to moisture which can damage the plates.

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

## ⚠ Disposal

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

## Instructions for Use

### Sample Preparation

1. Use appropriate sterile diluents:

Butterfield's phosphate buffered dilution water<sup>1</sup>, 0.1% peptone water<sup>1,2</sup>, peptone salt diluent<sup>2</sup>, quarter-strength Ringer's solution, dipotassium hydrogen phosphate, saline solution (0.85-0.90%)<sup>2</sup>, bisulfite-free letheen broth, or distilled water.

**Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm HSCC 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 6.5 - 7.5. For acidic products, adjust the pH with 1N NaOH. For alkaline products, adjust the pH with 1N HCl.

## Plating

1. Place the 3M Petrifilm HSCC Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular to the inoculation area dispense 5 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™ High Sensitivity Spreader (Catalog #6481) on the center of the plate. Press gently on the center of the 3M Petrifilm High Sensitivity Spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm HSCC Plate growth area before the gel is formed. Do not slide the 3M Petrifilm High Sensitivity Spreader across the film.
5. Remove the spreader and leave the plate undisturbed for at least two to five minutes to permit the gel to form.

## Incubation

Incubate plates in a horizontal position with the clear side up in stacks of no more than 10 plates. Incubate 3M Petrifilm HSCC Plates 24 hours ± 2 hours. Several incubation times and temperatures can be used depending on current local reference methods, some of which are listed in the section below titled “**Specific Instructions for Validated Methods**”.

## Interpretation

1. 3M Petrifilm HSCC Plates can be counted using a standard colony counter or other illuminated magnifier. Do not count artifact bubbles that may be present.

The interpretation of coliform colonies on the 3M Petrifilm HSCC Plate varies by method depending on current local reference methods, some of which are listed in the section below titled “**Specific Instructions for Validated Methods**”.

For example:

### **Enumeration of colonies associated with gas:**

Coliform colonies are red and closely associated (within one colony diameter) with entrapped gas.

### **Enumeration of colonies with or without gas for thermotolerant enumeration:**

After incubating the 3M Petrifilm HSCC Plates for 24 hours ± 2 hours at 44°C ± 1°C, thermotolerant coliforms are defined as red colonies with or without gas.

2. The circular growth area is approximately 60 cm<sup>2</sup>. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in two or more representative squares and determining the average number per square. Multiply the average number by 60 to determine the estimated count per plate.
3. When colonies are present in large numbers, 3M Petrifilm HSCC Plates will have a deepening of the gel color and either or both of the following characteristics: many small, indistinct colonies and/or many gas bubbles. 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.

For further information refer to the appropriate “3M™ Petrifilm™ High Sensitivity Coliform Count Plate Interpretation Guide.”

## **Specific Instructions for Validated Methods**

**AOAC® Official Methods<sup>SM</sup>** (996.02 Coliform Count in Dairy Products, High-Sensitivity Dry Rehydratable Film Method)

Incubate 3M Petrifilm HSCC Plates 24 hours ± 2 hours at 32°C ± 1°C.

## **NF Validation by AFNOR Certification**

**NF Validation certified method in compliance with ISO 16140<sup>5</sup> in comparison to ISO 4831<sup>3</sup> (3M-01/7-03/99)**

Use the following details when implementing the above Instructions for Use:

**Scope of the validation:** human food products.

### **Sample preparation:**

Use only ISO listed diluents<sup>4</sup>.

**Incubation:**

Incubate 3M Petrifilm HSCC Plates 24 hours  $\pm$  2 hours at 30°C  $\pm$  1°C or 37°C  $\pm$  1°C.

**Interpretation:**

Coliforms are defined as red colonies with gas on 3M Petrifilm HSCC Plates. Calculate the number of microorganisms present in the test sample according to ISO 7218<sup>8</sup> for one plate per dilution. Estimates are outside of the scope of the NF Validation Certification.



**3M-01/7-03/99**  
**Alternative Analytical methods for**  
**Agribusiness**

For more information about end of validity, please refer to NF VALIDATION certificate available on the website mentioned above.

**References**

1. FDA. Bacteriological Analytical Manual (BAM), 8th Edition, Revision A, 1998.
2. ISO 6887: Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
3. ISO 4831: Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of coliforms – Most probable number technique.
4. ISO 4832: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – colony-count technique.
5. ISO 16140: Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.
6. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
7. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
8. ISO 7218: Microbiology of food and animal feeding stuffs – General rules for microbiological examination.

Refer to the current versions of the standard methods listed above.

**Explanation of Symbols**

AOAC is a registered trademark of AOAC INTERNATIONAL  
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