

## Product Instructions

-  (EN) Rapid Coliform Count Plate
-  (FR) Test pour la numération rapide des Coliformes
-  (DE) Schnellenachweis Coliforme
-  (IT) Piastra per il conteggio rapido dei Coliformi
-  (ES) Placa para recuento rápido de bacterias coliformes
-  (NL) Snelle Coliform Telplaat
-  (SV) Snabb odlingsplatta för koliforma bakterier
-  (DA) Rapid Coliform Tælleplade
-  (NO) Hurtigfilm for koliforme bakterier
-  (FI) Nopea koliformien kasvatusalusta
-  (PT) Placa para Contagem Rápida de Coliformes
-  (EL) Πλακίδιο Ταχείας Μέτρησης Κολοβακτηριδίων
-  (PL) Płytko do szybkiego oznaczania liczby Coliform
-  (HU) Gyors Coliform táptalaj
-  (CS) Plotna pro rychlé stanovení počtu koliformních bakterií
-  (RO) Placă pentru numărare rapidă bacterii coliforme
-  (RU) Тест-пластина для экспресс- подсчёта колиформных бактерий
-  (TR) Hızlı Koliform Sayım Plakası
-  (JA) 大腸菌群数迅速測定用プレート (RCCプレート)
-  (ZH) 快速大肠菌群测试片
-  (TH) แผ่นอาหารเลี้ยงเชื้อสำหรับนับจำนวนโคลิฟอร์มแบบรวดเร็ว
-  (KO) 속성대장균군 측정용 플레이트



## Product Instructions

# Rapid Coliform Count Plate

### Product Description and Intended Use

The 3M™ Petrifilm™ Rapid Coliform Count (RCC) Plate is a sample-ready-culture-medium system which contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, a pH indicator to detect acid and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm RCC Plates are useful for the enumeration of coliform bacteria in the food and dairy industries. 3M Petrifilm RCC Plate components are decontaminated though not sterilized. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

AOAC INTERNATIONAL and the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) define coliforms as Gram-negative rods, which produce acid and gas from lactose fermentation. As colonies grow on the 3M Petrifilm RCC Plate and produce acid, the pH indicator in the plate changes from red-orange to yellow, providing a presumptive indication of coliforms. Gas trapped around coliform colonies indicates confirmed coliforms.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832<sup>1</sup>, enumerating coliforms by the colony-count technique, defines coliforms by colony size and acid production on VRB with lactose (VRBL) agar. On 3M Petrifilm RCC Plates these acid-producing coliforms are indicated by yellow acid zones, or red colonies with or without gas. ISO method 4831<sup>2</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 RCC Plates these coliforms are indicated by red colonies associated with gas. AFNOR Certification has certified 3M Petrifilm RCC Plates as a method in comparison to ISO method 4831<sup>2</sup> and ISO method 4832<sup>1</sup>.

### Safety

The user should read, understand, and follow all safety information in the instructions for the 3M Petrifilm RCC 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 instruction contained in the instructions for use.
- Do not use beyond expiration date.

#### To Reduce risk associated with bacterial infection and workplace contamination:

- Perform 3M Petrifilm RCC testing in a properly equipped laboratory under the control of a skilled microbiologist.
- The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices<sup>3</sup>, ISO 7218<sup>4</sup>, or ISO 17025<sup>5</sup>.

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

- 3M has not documented 3M Petrifilm RCC Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm RCC Plates for testing water, pharmaceuticals, or cosmetics. Acceptance of the 3M Petrifilm RCC Plate method for the testing of water per an accepted local government regulation is at the sole discretion and responsibility of the end-user.
- 3M Petrifilm RCC Plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of coliform or other bacteria.
- 3M Petrifilm RCC Plates do not differentiate any one coliform strain from another.
- Do not use 3M Petrifilm RCC Plates in the diagnosis of conditions in humans or animals.

Consult the Material Safety Data Sheet for additional information.

For information on documentation of product performance, visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.



## User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or distributor for more 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. The food sample itself may influence results. For example, foods with high sugar content may increase the potential for gas production from non-coliform *Enterobacteriaceae*.

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. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

## 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 and Disposal

Store **unopened** 3M Petrifilm RCC Plate pouches refrigerated or frozen at temperatures less than or equal to 8°C (46°F). Just prior to use, allow unopened 3M Petrifilm RCC Plate pouches to come to room temperature before opening (20-25°C / <60% RH). Return unused 3M Petrifilm RCC 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 one month.

It is recommended that resealed pouches of 3M Petrifilm RCC 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 RCC Plates in a sealable container. To remove frozen 3M Petrifilm RCC 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 RCC 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 RCC Plates to moisture which can damage the plates.

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

## △ Disposal

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

## Instructions for Use

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

### Sample Preparation

1. Use appropriate sterile diluents:

Butterfield's phosphate buffer<sup>6</sup>, 0.1% peptone water<sup>7</sup>, peptone salt diluent<sup>6,7</sup> saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water. See section, "Specific Instructions for Validated Methods" for specific requirements.



**Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm RCC 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 (104-113°F).

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

### Incubation

1. Incubate 3M Petrifilm RCC 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 section below titled **Specific Instructions for Validated Methods**.
2. Examine 3M Petrifilm RCC Plates for coliform growth at any time during a 24 hours ± 2 hours incubation interval depending on the desired information and method being followed (described below\*). See section, "Specific Instructions for Validated Methods" for specific requirements. Because coliform growth is affected by temperature, time out of the incubator should be minimized to avoid extending the detection time.

### Interpretation and Enumeration

For interpretation see section "Specific Instructions for Validated Methods"

1. Indirect back lighting may enhance early detection of yellow acid zones on 3M Petrifilm RCC Plates. Coliform colonies may begin to appear at 6 hours of incubation as discrete yellow zones indicating colony forming units (CFUs) (see Figure E). Continue incubating 3M Petrifilm RCC Plates to detect additional acid zones and/or red colonies associated with acid. 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.

Some coliforms produce large amounts of acid. For these organisms, fusion of the yellow acid zones could occur at about 20 colonies per plate. The circular growth area is approximately 20 cm<sup>2</sup>. Estimates can be made on 3M Petrifilm RCC Plates containing greater than 50 acid zones by counting the number of acid zones in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine total count per 3M Petrifilm RCC Plate.

2. Where necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel (see Figure H). Test using standard procedures.
3. If the 3M Petrifilm RCC 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 minus 15°C (5°F) for no longer than one week.

For further information refer to the 3M™ Petrifilm™ Rapid Coliform Count Plate "Interpretation Guide." If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

### Specific Instructions for Validated Methods

**AOAC® Official Methods<sup>SM</sup> 2000.15:** Dry Rehydratable Film Method for the Rapid Enumeration of coliform in Foods.

Incubate 3M Petrifilm RCC Plates up to 24 hours ± 2 hours at 35°C ± 1°C.

### NF Validation by AFNOR Certification:

**NF Validation certified method in compliance with ISO 16140<sup>8</sup> in comparison to ISO 4832<sup>1</sup> (3M-01/5-03/97 A).**

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

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

**Sample preparation:**

Use only ISO listed diluents<sup>7</sup>.

**Incubation:**

For processed pork products and seafood incubate 3M Petrifilm RCC Plates 14 hours  $\pm$  30 minutes at 30°C  $\pm$  1°C.

For all other foods incubate 3M Petrifilm RCC Plates 14 hours  $\pm$  30 minutes at 35°C  $\pm$  1°C.

**Interpretation:**

Calculate the number of microorganisms present in the test sample according to ISO 7218<sup>4</sup> for one plate per dilution. Estimates are outside of the scope of the ISO 16140<sup>8</sup> validation.

**NF Validation certified method in compliance with ISO 16140<sup>8</sup> in comparison to ISO 4832<sup>1</sup> (3M-01/5-03/97 B).**

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

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

**Sample preparation:**

Use only ISO listed diluents.<sup>1</sup>

**Incubation:**

For processed pork products and seafood incubate 3M Petrifilm RCC Plates 24 hours  $\pm$  2 hours at 30°C  $\pm$  1°C.

For all other foods incubate 3M Petrifilm RCC Plates 24 hours  $\pm$  2 hours at 35°C  $\pm$  1°C.

**Interpretation:**

Calculate the number of microorganisms present in the test sample according to ISO 7218<sup>4</sup> for one plate per dilution. Estimates are outside of the scope of the ISO 16140<sup>8</sup> validation.

**NF Validation certified method in compliance with ISO 16140<sup>8</sup> in comparison to ISO 4831<sup>2</sup> (3M-01/5-03/97 C).**

**Scope of the validation:** All human food products, except processed pork products.

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

**Sample preparation:**

Use only ISO listed diluents.<sup>7</sup>

**Incubation:**

For seafood products incubate 3M Petrifilm RCC Plates 24 hours  $\pm$  2 hours at 30°C  $\pm$  1°C.

For all other foods incubate 3M Petrifilm RCC Plates 24 hours  $\pm$  2 hours at 35°C  $\pm$  1°C.

**Interpretation:**

Calculate the number of microorganisms present in the test sample according to ISO 7218<sup>4</sup> for one plate per dilution. Estimates are outside of the scope of the ISO 16140<sup>8</sup> validation.

**NF VALIDATION by AFNOR Certification**



**3M 01/05-03/97 A, B and C**  
**ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS**  
**[www.afnor-validation.com](http://www.afnor-validation.com)**

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



## References

1. ISO 4832: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms--- Colony count technique.
2. ISO 4831: Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms--- Most probable number technique.
3. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
4. ISO 7218: Microbiology of food and animal feeding stuffs - General rules for microbiological examination.
5. ISO 17025: General requirements for the competence of testing and calibration laboratories.
6. FDA. Bacteriological Analytical Manual (BAM), Reagents Index for BAM found at: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm055791.htm>.
7. ISO 6887: Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
8. ISO 16140: Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods.

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## Explanation Of Symbols

[www.3M.com/foodsafety/symbols](http://www.3M.com/foodsafety/symbols)



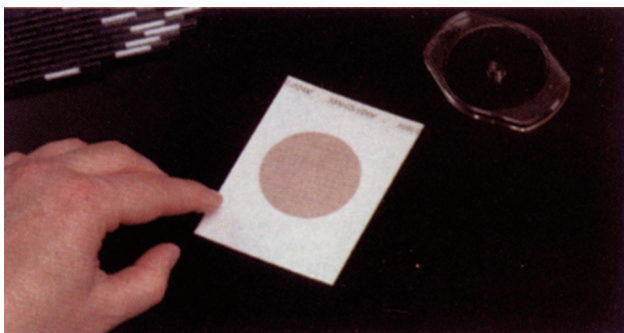


Figure A.

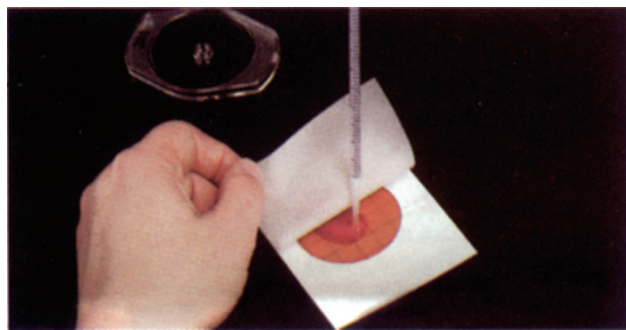


Figure B.

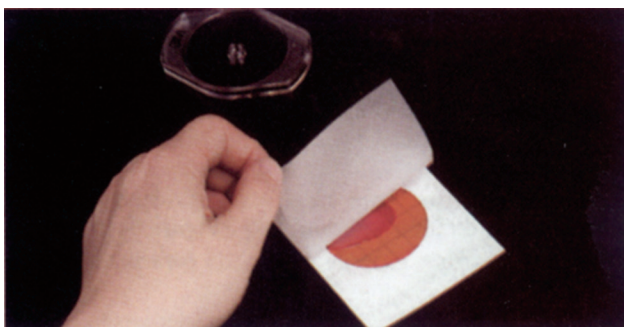


Figure C.

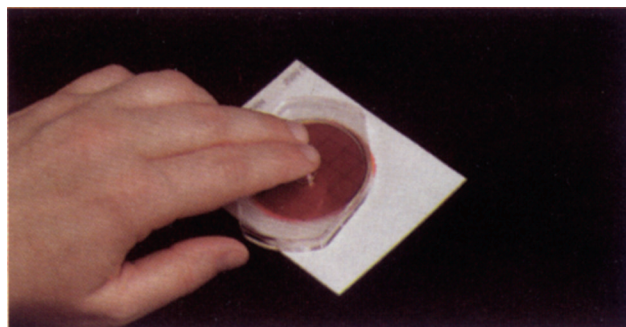


Figure D.

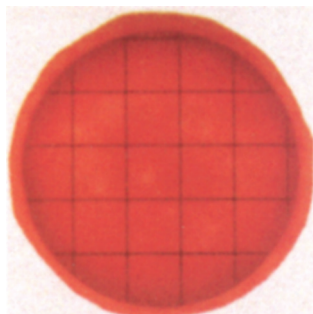


Figure E.

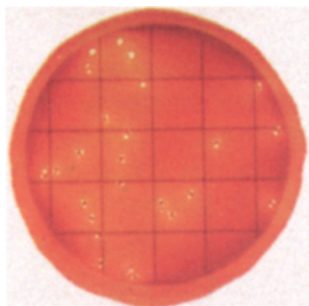


Figure F.

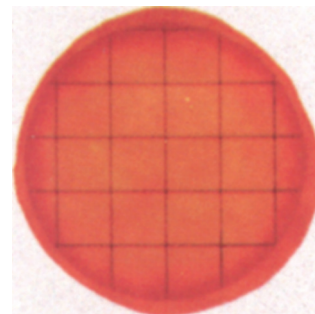


Figure G.

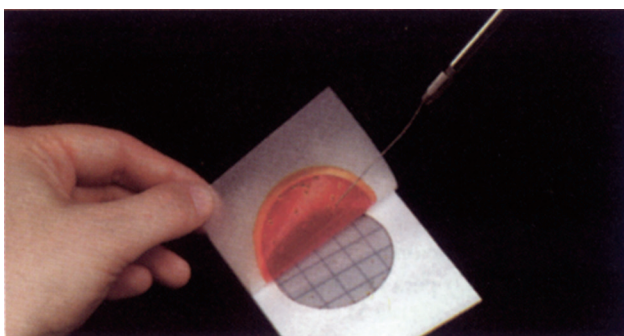


Figure H.

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