

**Storage**

1. Refrigerate unopened packages of Petrifilm count plates. Use before expiration date on package.

2. To seal opened package, fold end over and secure with tape or a clip.

3. Keep resealed package at ≤21°C (≤70°F), ≤50%RH. Do not refrigerate opened packages. Use Petrifilm count plates within one month after opening.

**Sample Preparation**

4. Prepare a 1:10 or greater dilution of food product. Weigh or pipette food product into a stomacher bag, dilution bottle, or other appropriate sterile container.

5. Add appropriate quantity of diluent. These include Standard Methods phosphate buffer, 0.1% peptone water, distilled water, phosphate buffered saline, and Butterfield’s buffer. Do not use buffers containing sodium citrate or thiosulfate.

6. Blend or homogenise sample as per current procedure.

**Inoculation**

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

8. With pipette perpendicular to Petrifilm plate, place 1 mL of sample onto centre of bottom film.

9. Release top film; allow it to Drop. Do not roll top film down.
### Inoculation

10. With ridge side down, place spreader on top film over inoculum.

11. Gently apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.

12. Lift spreader. Wait one minute for gel to solidify.

### Incubation

13. Incubate Petrifilm count plates with the clear side up in stacks of 20 or less, at a temperature of 30°C +/- 1°C for 48 +/- 2 hours (for all products dairy and raw shellfishes excepted) or for 72 +/- 2 hours for all products.

### Interpretation

14. Read colonies. A colony counter or any other magnifier light source can be used. Refer to Guide to Interpretation when reading results.

### Additional Comments

- Steps 9 and 10 are unique to Petrifilm Aerobic count plates.
- Note: Remember to inoculate and spread each Petrifilm count plate before going on to the next.
3M™ Petrifilm™ Interpretation Guide

3M™ Petrifilm™ Aerobic Count Plates
As with an agar pour plate, the preferable counting range on a Petrifilm Aerobic count plate is 10-300 colonies. See figure 4.

Estimated count = 420

When colonies number more than 300 as in figure 5, estimate the count. Determine the average number of colonies in one square (1 cm²) and multiply it by 20 to obtain the total count per count plate. The inoculated area on a Petrifilm Aerobic count plate is approximately 20 cm².
Count = TNTC
Figure 6 shows a Petrifilm Aerobic count plate with colonies that are too numerous to count (TNTC).

Count = TNTC
With very high counts, the entire growth area may turn pink, as shown in figure 7. You might observe individual colonies only at the edge of the growth area. Record this as a TNTC result.

Count = TNTC
Occasionally, distribution of colonies appears uneven as shown in figure 8. This is also an indication of a TNTC result. In fact, the distribution is even.

Count = TNTC
The colonies on the Petrifilm Aerobic count plate in figure 9 appear countable at first glance. However, when you look closely at the edges of the growth area, you can see a high concentration of colonies. Record this as a TNTC result.
Estimated count = 160
A few species of bacteria liquify the gel in the Petrifilm Aerobic count plate, as shown in figure 10. When this occurs, determine the average count in a few unaffected squares and then estimate the total count. Do not count red spots within the liquified area.

Count = 83
Colonies on Petrifilm Aerobic count plates are red and can be easily distinguished from opaque food particles that may cause confusion with agar pour plates. See figure 11.
**3M™ Petrifilm™ Enterobacteriaceae Count 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.

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**Reminders for use**

1. **Refrigerate unopened** packages at ≤ 8°C. Use before expiration date on package.
2. To seal opened pouch, fold end over and tape shut.
3. Keep resealed packages at ≤ 25°C (≤ 77°F), ≤ 50% RH. Do not refrigerate opened packages. Use Petrifilm count plates within one month after opening.

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**Storage**

- place petrifilm EL plate on level surface.
- lift top film.
- with pipette perpendicular to petrifilm count plate, place 1 mL of sample onto centre of bottom film.
- carefully roll top film down to avoid trapping air bubbles. Do not let top film drop.

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**Sample Preparation**

- Weigh or pipette food product into a sterile container such as a bag or a bottle, in order to obtain the appropriate dilution.
- add appropriate quantity of one of the sterile diluents recommended as diluents for general use in ISO 6887 or ISO 8261/IDF 122 such as peptone salt diluent and buffered peptone water. Other diluents may also be used such as, for example, dipotassium hydrogen phosphate solution (ISO 8261 /IDF122122) or sodium bisulphate-free letheen broth or distilled water.
- blend or homogenise sample as per current procedure.

**Adjust pH of the diluted sample to between 6.5 and 7.5 :**
- for acid products, use NaOH 1N,
- for alkaline products, use HCl 1N.

Or use a buffer diluent to reach this range of pH.

**Do not use buffers containing citrate, bisulphite or thiosulphate as they may inhibit growth.**

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**Inoculation**

- place Petrifilm EL plate on level surface. Lift top film.
- with pipette perpendicular to Petrifilm count plate, place 1 mL of sample onto centre of bottom film.
- carefully roll top film down to avoid trapping air bubbles. Do not let top film drop.
Inoculation

10 **With flat** side down, place spreader on top film over inoculum.

11 **Gently** apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.

12 Lift spreader. Wait one minute for gel to solidify.

Incubation

13 Incubate Petrifilm count plates with the clear side up in stacks of 20 or less, at a temperature of 35°C ± 1°C or 37°C ± 1°C for 24 ± 2 hours.

Interpretation

14 Read colonies. A colony counter or any other magnifier light source can be used. Refer to interpretation guide section when reading results.

15 To isolate colonies for further identification, lift top film and pick the colony from the gel.

Additional Comments

• Note: Remember to inoculate and spread each Petrifilm count plate before going on to the next.
Enterobacteriaceae count = 13

It is easy to count Enterobacteriaceae colonies on a Petrifilm Enterobacteriaceae count plate. A red indicator dye in the plate colours all colonies, and the top film traps gas if it is produced by the bacteria. The acid producing bacteria are seen as red colonies surrounded by a yellow zone associated with acid production by the pH indicator in the medium.

Enterobacteriaceae colonies have the following characteristics on Petrifilm Enterobacteriaceae count plate: Enterobacteriaceae can produce colonies which are associated with gas bubbles only (see figure 1, circle 1). Enterobacteriaceae can also produce red colonies with acid zones only (see figure 1, circle 2). Finally, Enterobacteriaceae produce red colonies which are associated with an acid zone and gas bubbles (see figure 1, circle 3).

Circles 1 and 3 in figure 1 also show how bubble patterns can vary. Sometimes gas disrupts the colony so that the colony „outlines“ the bubble as in circle 3.

Enterobacteriaceae count = 9

Figure 2 shows a Petrifilm Enterobacteriaceae count plate.
Enterobacteriaceae count = 77
The recommended counting range on Petrifilm Enterobacteriaceae count plates is 15 - 100 colonies. Samples having counts greater than 100 Enterobacteriaceae per plate are to be estimated as having counts greater than 100 times the dilution. See figure 5.

Enterobacteriaceae count = 0
Notice the change in gel colour in figures 3 through 8. As the Enterobacteriaceae count increases, the colour of the gel lightens from purple to yellow or cream coloured.

Enterobacteriaceae count = TNTC (estimated)
Petrifilm Enterobacteriaceae count plate with colonies too numerous to count (TNTC) have caused lightening of the gel colour, plus one or two of the following features:
1) many small colonies, or, 2) many gas bubbles. See figure 6.
Enterobacteriaceae count = TNTC (estimated)
In figure 7, the count is so high that acid zones and gas bubbles are not easily seen. A lightening of the gel colour indicates that the result is TNTC.

Enterobacteriaceae count = TNTC (estimated)
Petrifilm Enterobacteriaceae count plate in figure 8 has two features indicating TNTC colonies:
1) lightening of the gel colour, and
2) many small colonies.

Enterobacteriaceae count = 44
Artefact bubbles may result from improper inoculation of Petrifilm Enterobacteriaceae count plate. They are irregularly shaped, and not associated with a red colony. See figure 9.

Enterobacteriaceae count = 2
Food particles are often irregular or filamentous shaped and not associated with gas bubbles or acid zones. See figure 10.
Enterobacteriaceae count = 29

Food particles can also be seen as dark points but are not associated with gas bubbles or acid zones. See figure 11.
Esta guía sirve para familiarizarse con los resultados obtenidos en las placas 3M™ Petrifilm™ para Recuento de Coliformes (CC). Para más información, contactar con el distribuidor oficial de Productos 3M Microbiology.

Las placas Petrifilm CC contienen los nutrientes del Violeta Rojo Bilis (VRB) modificado, un agente gelificante soluble en agua fría y un indicador de tetrazolio que facilita la enumeración de colonias. El film superior atrapa el gas producido por la fermentación de la lactosa por los coliformes.

- La ISO define los coliformes por su capacidad de crecer en medios específicos y selectivos. El método ISO 4832, que enumera los coliformes por la técnica del recuento de colonias, define los coliformes por el tamaño de las colonias y la producción de ácido en el Agar VRB con lactosa (VRBL). En las placas Petrifilm CC, estos coliformes productores de ácido se muestran como colonias rojas con o sin gas (ver Círculo 1). El método ISO 4831, que enumera los coliformes por el método del Número Más Probable (NMP), define los coliformes por su capacidad de crecer y producir gas a partir de la lactosa en un caldo selectivo. En las placas Petrifilm CC, estos coliformes se muestran como colonias rojas asociadas a gas (ver Círculo 2).

- La AOAC INTERNATIONAL y la FDA (Food and Drug Administration) / BAM definen los coliformes como bacilos Gram negativos que producen ácido y gas a partir de la lactosa durante la fermentación metabólica. Las colonias de coliformes que crecen en las placas Petrifilm CC producen ácido que provoca que el indicador de pH oscurezca el color del gel; el gas atrapado alrededor de las colonias indica coliformes (ver Círculo 2).

El tiempo y temperatura de incubación, así como la interpretación de las placas Petrifilm CC puede variar con el método.

La AOAC®, la AFNOR y la NMKL han validado el uso de las placas Petrifilm CC bajo condiciones específicas. Ver páginas 2 y 3 de esta Guía de Interpretación.

Recuento de colonias productoras de gas : 75
Recuento de colonias no productoras de gas : 24
Recuento total : 99
Interpretación de las Placas 3M Petrifilm CC según los protocolos descritos por las siguientes organizaciones: AOAC®, NMKL y AFNOR

**Lectura según los AOAC®, Official Methods**
(986.33, 989.10 y 991.14)

Incubación :
- Enumeración de coliformes en leche, leche cruda y productos lácteos (Métodos Oficiales 986.33 y 989.10) : incubar 24h +/- 2h a 32°C +/- 1°C.
- Enumeración de coliformes en todos los productos, excepto los arriba mencionados (Método Oficial 991.14) : incubar 24h +/- 2h a 35°C +/- 1°C.

Interpretación :
- Coliformes : Contar todas las colonias rojas con gas.

**Lectura según el método validado por la NMKL**
(147.1993)

Incubación :
24h +/- 2h a 37°C +/- 1°C

Interpretación :
- Coliformes : Contar todas las colonias rojas con gas.
97 coliformes, método aprobado AFNOR comparado con el método ISO 4832
72 coliformes productores de gas, método aprobado AFNOR comparado con el método ISO 4831.

Lectura según la aprobación AFNOR para coliformes totales
(certificados número 3M 01/2-09/89A y 3M 01/2-09/89B)
Incubación :
24h +/- 2h a 30°C +/- 1°C
Interpretación :
• Comparación con el método ISO 4832 (certificado 3M 01/2-09/89A) :
  Contar todas las colonias rojas con o sin gas
• Comparación con el método ISO 4831 (certificado 3M 01/2-09/89B) :
  Contar sólo las colonias rojas con gas.

21 coliformes, método aprobado AFNOR comparado con el método NF V08-017.

Lectura según la aprobación AFNOR para coliformes termotolerantes
(certificados número 3M 01/2-09/89C)
Incubación :
24h +/- 2 a 44°C +/- 1°C
Interpretación :
• Comparación con el método NF V08-017 :
  Contar todas las colonias rojas con o sin gas.
Recuento de colonias  = 0
Las burbujas de fondo son una característica del gel y no resultado del crecimiento de coliformes. Las burbujas de fondo son pequeñas o puntiformes y no tienen una colonia asociada.

Recuento de colonias no productoras de gas : 7
Recuento de colonias productoras de gas : 8
Recuento total : 15
La Figura 3 muestra como la forma de las burbujas puede variar. Algunas veces el gas deforma la colonia y hace que la colonia "perfile" la burbuja (ver Círculos 1 y 2). Estas burbujas de gas tienen aproximadamente el diámetro de una colonia.

Pueden aparecer burbujas como artefactos debidos a una inoculación inadecuada de la placa Petrifilm CC o de aire atrapado en la muestra. Las burbujas tienen forma irregular y no están asociadas a una colonia. (ver Círculo 3).

Recuento de colonias productoras de gas : 29
Recuento de colonias no productoras de gas : 83
Recuento total : 112
El intervalo óptimo de recuento (colonias totales) en las placas Petrifilm CC es 15 - 150 colonias.
No contar las colonias que aparecen sobre la zona blanca ya que no están bajo la influencia selectiva del medio (ver Círculo 1).

Recuento total estimado : 310
El área de crecimiento circular de la placa Petrifilm CC es de aproximadamente 20 cm2. Se pueden hacer estimaciones en placas con más de 150 colonias contando el número de colonias en uno o varios cuadrados representativos y obteniendo el promedio. Multiplicar dicho número por 20 para obtener el recuento estimado por placa Petrifilm CC.

Para obtener un recuento más preciso, diluir más la muestra.
Placas TNTC Demasiado Numerosas Para Contar

Para obtener un recuento más preciso, diluir más la muestra.

Placas TNTC (Demasiado Numerosas Para Contar)
Las placas Petrifilm CC con colonias TNTC tienen una o más de las características siguientes: muchas colonias pequeñas, muchas burbujas de gas, y un oscurecimiento del color del gel.

Colonias productoras de gas : 4
Cuando un alto número de microorganismos no-coliformes, tales como Pseudomonas, están presentes en las placas Petrifilm CC, el gel puede virar a amarillo.

Burbujas

Colonias productoras de gas : 2
Las partículas alimenticias tienen forma irregular y no están asociadas a burbujas de gas (ver Círculo 1).

Arriba se muestran varios ejemplos de burbujas asociadas a una colonia. Todos ellos se deben contar.
**Instrucciones de uso**

**Almacenamiento**

1. **Conservar** las bolsas cerradas a ≤ 8°C. Usar antes de la fecha de caducidad impresa en la bolsa. En zonas con alta humedad donde puede haber condensación, es mejor dejar que las bolsas alcancen la temperatura ambiente antes de abrirlas.

2. Para cerrar las bolsas que se están utilizando, doblar los extremos y cerrarlos con celo.

3. Mantener las bolsas una vez cerradas a ≤ 25°C, a HR <50%. **No refrigerar las bolsas abiertas.** Usar las placas Petrifilm en un mes desde su apertura.

**Preparación de la muestra**

4. Pesar o pipetear el producto alimenticio en un contenedor estéril adecuado, como una bolsa tipo Stomacher, frasco de dilución, bolsa Whirl-Pak®, o cualquier otro contenedor estéril.

5. Si es necesario, utilizar diluyentes **estériles** apropiados: agua peptona sal (método ISO 6887) (Diluyente de Máxima Recuperación), tampón fosfato de Butterfield (tampón fosfato IDF, KH₂PO₄ a 0.0425g/l, ajustar pH a 7.2), agua peptonada al 0.1%, agua peptonada tamponada (método ISO 6579), solución salina (0.85 - 0.90%), caldo letheen sin bisulfito, o agua destilada.

6. Mezclar u homogeneizar la muestra según el procedimiento habitual.

**Ajustar el pH de la muestra diluida entre 6.6 y 7.2:**
- para productos ácidos, usar NaOH 1N,
- para productos alcalinos, usar HCl 1N.

No usar tampones que contengan citrato, bisulfito o tiosulfato, ya que pueden inhibir el crecimiento.
**Inoculación**

7 Colocar la placa Petrifilm en una superficie **plana**. Levantar el film superior. Con una pipeta colocada de forma **perpendicular** a la placa Petrifilm, colocar 1 ml. de la muestra en el centro del film inferior.

8 Bajar el film superior **con cuidado** evitando introducir burbujas de aire. **No** dejarlo caer.

9 Con la cara **lisa** hacia abajo, colocar el aplicador en el film superior sobre el inóculo. **Con cuidado**, ejercer una presión sobre el aplicador para repartir el inóculo sobre el área circular antes de que se forme el gel. **No** girar ni deslizar el aplicador. Levantar el aplicador. Esperar al menos un minuto a que solidifique el gel.

**Incubación**

10 Incubar las placas cara arriba en pilas de hasta 20 placas. El tiempo e incubación varía según el método.

**Interpretación**

11 Las placas Petrifilm pueden leerse con un contador de colonias standard u otra lente de aumento iluminada. Para leer los resultados, consultar la Guía de Interpretación.

12 Las colonias pueden aislarse para una posterior identificación. Levantar el film superior y seleccionar la colonia del gel.

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**Métodos aprobados más usuales:**

**Coliformes totales**
- Métodos Oficiales 986.33 y 989.10 (leche, leche cruda, otros productos lácteos): Incubar 24h ± 2h a 32°C ± 1°C.
- Método Oficial AOAC 991.14 (todos los alimentos): Incubar 24h ± 2h a 35°C ± 1°C.
- Método NMKL 147.1993: Incubar 24h ± 2h a 37°C ± 1°C.
- Métodos validados AFNOR 3M 01/2-09/89A y B: Incubar 24h ± 2h a 30°C ± 1°C.

**Coliformes termotolerantes (fecales)**
- Método validado AFNOR 3M 01/2-09/89C: Incubar 24h ± 2h a 44°C ± 1°C.

Para esta alta temperatura, es necesario una humidificación del incubador.
This guide should familiarize you with results on 3M™ Petrifilm™ E. coli and Coliform Count plates (EC). For more information contact the official 3M Microbiology Products representative in your area.

Petrifilm EC plates contain VRB nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity BCIG, and a tetrazolium indicator that facilitates colony enumeration. The top film traps gas produced by the lactose fermenting Coliforms and E. coli.

**E. coli**

- Time and Temperature of incubation as well as interpretation of the Petrifilm EC plates vary by method. Therefore, this may give slightly different results. Temperatures of incubation used in the most common methods are mentioned in the package insert, and examples are shown in the technical section (pages 2 and 3) of this Guide.
- Do not count colonies on the foam dam since they are removed from the selective influence of the medium.
- Follow the time and temperature usually used in the laboratory. These are the most common used temperatures (E. coli and coliform): 35, 37, 42 or 44°C during 24 to 48h.

E. coli are able to grow on media containing Violet Red Bile (VRB) nutrients. Most E. coli (about 97%) produce beta-glucuronidase which reacts with a BCIG indicator dye in the Petrifilm EC plate that makes the colony turn blue to red-blue. About 95% of E. coli produce gas from lactose, this is indicated by colonies associated (within approximately one colony diameter) with entrapped gas. See Circle 1. E. coli colonies appear blue to red-blue and produce gas, confirm blue to red-blue colonies without gas. See Circle 2. In some validation processes, this interpretation has been modified: the following organizations AOAC, NORDVAL, EMMAS have validated or assessed the use of the Petrifilm EC plates under specific conditions. See pages 2 and 3 of this Interpretation Guide.

**Coliforms**

The Petrifilm EC plates can also be used to search for Coliforms.

- **ISO** defines Coliforms by their ability to grow in method-specific, selective media. **ISO method 4832**, enumerating Coliforms by the colony count technique, defines Coliforms by colony size and acid production on VRB with lactose (VRBL) agar. On Petrifilm EC plates, these acid-producing Coliforms are indicated by red colonies with or without gas (within approximately one colony diameter). See Circle 3. **ISO method 4831**, 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 Petrifilm EC plates these Coliforms are indicated by red colonies associated (within approximately one colony diameter) with gas. See Circle 4.

- **AOAC INTERNATIONAL** and U.S. FDA Bacteriological Analytical Manual (BAM) define Coliforms as Gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm EC plate produce acid which deepen the gel color. Gas trapped around Coliform colonies (within approximately one colony diameter) indicates confirmed Coliforms. See Circle 4.

Remark:
Because most E. coli 0157:H7 strains are atypical: they do not grow at temperatures ≥ 44.5°C, are glucuronidase negative, and therefore will not produce a blue precipitate. They will appear as non-E. coli Coliforms (red with gas).
Interpretations of 3M™ Petrifilm™ EC Plates

Recommended method in France

**Incubation:**
- 24h +/- 2h at 42°C +/- 1°C

**Interpretation:**
- *E. coli*: Count all blue colonies with and without gas.

Reading following AOAC.
International all foods (method 991.14)

**Incubation:**
- Coliforms in all foods: incubate 24h +/- 2h at 35°C +/- 1°C.
- Enumeration of *E. coli* in all foods, except those here under:
  incubate 48h +/- 2h at 35°C +/- 1°C.

Reading following AOAC International, meat, poultry and seafood (method 998.08)

**Incubation:**
- Enumeration of *E. coli* in Meat, Poultry and Seafood, and Coliforms in all foods: incubate 24h +/- 2h at 35°C +/- 1°C.

**Interpretation (Methods 991.14 and 998.08):**
- *E. coli*: blue colonies with gas.
- Confirmed Coliforms: all colonies with gas (blue and red).
Reading following NORDVAL validated method (certificate n° 14)

**Incubation:**
- 37°C +/- 1°C

**Interpretation:**
- *E. coli*: Count all blue colonies, with and without gas after 48h +/- 2h of incubation.
- *Coliforms*: Count red colonies with gas and all blue colonies with or without gas after 24h +/- 2h of incubation.

Reading following EMMAS assessed method

**Incubation:**
- 48h +/- 2h at 37°C +/- 1°C

**Interpretation:**
- *E. coli*: Count all blue colonies with and without gas.
  *It is advisable to confirm blue colonies without gas, particularly when they are present in high proportion.*
3M™ Petrifilm™ E. coli and Coliform Count Plates

Notice the change in gel colour in figures 2 through 8. As the *E. coli* or Coliform count increases, the colour of the gel turns to dark red or purple-blue.

No growth

*E. coli count = 0*

Background bubbles are a characteristic of the gel and are not a result of *E. coli* or Coliform growth. Background gas bubbles are small to pin-point in size, regular in shape and do not have a colony associated with them. See Square 1.

*E. coli count = 13*

Gas producing Coliforms count = 28

As with VRB agar plates, the preferable counting range (total colony population) on Petrifilm EC plates is 15 - 150.

Do not count colonies that appear on the foam dam since they are removed from the selective influence of the medium. See Circle 1.

*E. coli count = 3*

Any blue in a colony (blue to red-blue) indicates the presence of *E. coli*. Front lighting may enhance the detection of blue precipitate formed by a colony.

- Circle 1 shows a red-blue colony using back lighting.
- Circle 2 shows the same colony with front lighting. The blue precipitate is more evident in this case.

*E. coli count = 20*

Estimated total count = 150

The Petrifilm EC plate circular growth area is approximately 20 cm². Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per Petrifilm EC plate.
Actual count ~ $10^8$

Petriﬁlm EC plates with colonies that are TNTC have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel colour from red to purple-blue.

Actual count ~ $10^8$

High concentrations of *E. coli* will cause the growth area to turn purple-blue.

Actual count ~ $10^4$

High concentrations of Coliforms (non *E. coli*) will cause the growth area to turn dark red. Additional dilutions are required to determine if *E. coli* are present.

Actual count ~ $10^4$

When high numbers of non-Coliforms organisms such as *Pseudomonas* are present on Petriﬁlm EC plates, the gel may turn yellow.
Food particles are irregularly shaped and are not associated with gas bubbles. See Circle 1.

Figure 11 shows how bubble patterns may vary. Sometimes gas disrupts the colony so that the colony “outlines” the bubble. See Circles 1 and 2. Artifact bubbles may result from improper inoculation of the Petrifilm EC plate or from trapped air within the sample. They are irregularly shaped and are not associated with a colony. See Circle 3.

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

The following are additional examples of various bubble patterns associated with a colony. All of them should be taken into account.
3M™ Petrifilm™ E. coli and Coliform Count Plates

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 packages at ≤8°C (≤46°F). Use before expiration date on package.
2. To seal opened package, fold end over and tape shut.
3. Keep resealed package at ≤25°C (≤77°F) and ≤50% RH. Do not refrigerate opened packages. Use Petrifilm plates within one month after opening.

Sample Preparation

4. Weigh or pipette food product into an appropriate sterile container such as stomacher bag, dilution bottle, Whirl-Pak® bag, or other sterile container.
5. Add appropriate quantity of one of the following sterile diluents: Butterfield’s phosphate buffer (IDF phosphate buffer, KH₂PO₄ at 0.0425g/L, adjust pH to 7.2), 0.1% peptone water, peptone salt diluent (ISO method 6887), saline solution (0.85 - 0.90%), or distilled water.

Sample Preparation

6. Blend or homogenize sample as per current procedure.

Inoculation

7. Place Petrifilm plate on level surface. Lift top film.
8. With pipette perpendicular to Petrifilm plate, place 1mL of sample onto center of bottom film.
9. Carefully roll top film down to avoid trapping air bubbles. Do not let top film drop.

Do not use buffers containing citrate, bisulfite or thiosulfate. Adjust pH of the diluted sample between 6.6 and 7.2:
- for acidic products, use NaOH 1N,
- for alkaline products, use HCl 1N.
**Inoculation**

10 With flat side down, place spreader on top film over inoculum.

11 Gently apply pressure on spreader to distribute over circular area. Do not twist or slide the spreader.

12 Lift spreader. Wait at least one minute for gel to solidify.

**Incubation**

13 Incubate plates with clear side up in stacks of up to 20. Incubation time and temperature vary by method*.

**Interpretation**

14 Petrifilm plates can be counted with a standard colony counter or other magnifier. Refer to the Interpretation Guide section when reading results.

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

**Most common approved methods:**

- AOAC Official Method 991.14: for coliforms, incubate 24h ± 2h at 35°C ± 1°C; for E. coli, incubate 48h ± 2h at 35°C ± 1°C.
- AOAC Official Method 998.08: for E. coli in Meat, Poultry and Seafood, and Coliforms in all foods, incubate 24h +/-2h at 35°C +/-1°C.
- NORDVAL approved method (certificate n° 14): for Coliforms, incubate 24h ± 2h at 37°C ± 1°C; for E. coli, incubate 48h ± 2h at 37°C ± 1°C.

* See product’s package insert.

**Additional Comments**

- Remember to inoculate and spread each Petrifilm plate before going on to the next plate.
- Incubation time and temperature vary by method, see product’s package insert.
Esta guía sirve para familiarizarse con los resultados obtenidos en las placas 3M™ Petrifilm™ Alta Sensibilidad para Recuento de Coliformes (HSCC). Para más información, contactar con el distribuidor oficial de Productos 3M Microbiology.

Las placas Petrifilm HSCC contienen un medio de cultivo selectivo listo para usar: Violeta Rojo Bilis (VRB), un agente gelificante soluble en agua fría y un indicador de tetrazolio que facilita la enumeración de colonias. El film superior atrapa el gas producido por la fermentación de la lactosa por los coliformes. El tiempo y la temperatura de incubación, así como la interpretación, varía según el método seguido.

• La ISO define los coliformes por su capacidad de crecer en medios específicos y selectivos. El método ISO 4832, que enumera los coliformes por la técnica del recuento de colonias, define los coliformes por el tamaño de las colonias y la producción de ácido en el Agar VRB con lactosa (VRBL). En las placas Petrifilm HSCC, estos coliformes productores de ácido se muestran como colonias rojas con o sin gas (separadas aproximadamente un diámetro de la colonia).

El método ISO 4831, que enumera los coliformes por el método del Número Más Probable (NMP), define los coliformes por su capacidad de crecer y producir gas a partir de la lactosa en un caldo selectivo. En las placas Petrifilm HSCC, estos coliformes se muestran como colonias rojas asociadas a gas (separadas aproximadamente un diámetro de la colonia).

• La AOAC INTERNATIONAL y la U.S. Food and Drug Administration (FDA) / Bacteriological Analytical Manual (BAM) definen los coliformes como bacilos Gram negativos que producen ácido y gas a partir de la lactosa durante la fermentación metabólica. Las colonias de coliformes que crecen en las placas Petrifilm HSCC producen ácido que oscurece el color del gel; el gas atrapado alrededor de dichas colonias indica coliformes (separadas aproximadamente un diámetro de la colonia).

Las placas Petrifilm HSCC están diseñadas para la detección de coliformes totales, y también coliformes termotolerantes (fecales).

Estas placas Petrifilm HSCC están especialmente recomendadas para detectar coliformes en bajo número en todos los alimentos.

La AFNOR ha validado el uso de las placas Petrifilm HSCC bajo condiciones específicas. Ver las Instrucciones de Uso de esta Guía de Interpretación.
Recuento de colonias productoras de gas: 82
Recuento de colonias no productoras de gas: 8
Recuento total: 90
La forma de las burbujas puede variar: ver Círculos 1 y 2.
Algunas veces, el gas producido deforma la colonia de coliformes y hace que la colonia “perfil” la burbuja.

Recuento total estimado: 320
El área de crecimiento circular es aproximadamente 60 cm². Se pueden hacer estimaciones en placas con más de 150 colonias contando el número de colonias en uno o varios cuadrados representativos y obteniendo el promedio. Multiplicar dicho número por 60 para obtener el recuento estimado por placa.

Para obtener un recuento más preciso, diluir más la muestra.

Placas TNTC (Demasiado Numeroso Para Contar)
Las placas Petrifilm HSCC con colonias TNTC tienen una o más de las características siguientes: muchas colonias pequeñas, muchas burbujas de gas, y un oscurecimiento del color del gel.

Para obtener un recuento más preciso, diluir más la muestra.

Colonias productoras de gas: 2
Las partículas alimenticias tienen forma irregular y no están asociadas a burbujas de gas (ver Círculo 1).
Pueden aparecer burbujas como artefactos debidas a una inoculación inadecuada de las placas Petrifilm HSCC. Las burbujas tienen forma irregular y no están asociadas a una colonia. (ver Círculo 2).
**Instrucciones de uso**

**3M™ Petrifilm™**

**Placas Alta Sensibilidad para Recuento de Coliformes**

Para Advertencias, Precauciones, Responsabilidad del Usuario, Garantía Limitada, Almacenamiento y Eliminación, e Instrucciones de Uso, ver el folleto del producto.

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### Almacenamiento

1. **Conservar** las bolsas cerradas a ≤8°C. Usar antes de la fecha de caducidad impresa en la bolsa. En zonas con alta humedad donde puede haber condensación, es mejor dejar que las bolsas alcancen la temperatura ambiente antes de abrirlas.

2. Para cerrar las bolsas que se están utilizando, doblar los extremos y cerrarlos con celo.

3. Mantener las bolsas una vez cerradas a ≤25°C. a HR ≤50%. **No refrigerar las bolsas abiertas.** Usar las placas Petrifilm en un mes desde su apertura.

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### Preparación de la muestra

4. Pesar o pipetear el producto alimenticio en un contenedor estéril adecuado, como una bolsa tipo Stomacher, frasco de dilución, bolsa Whirl-Pak®, o cualquier otro contenedor estéril.

5. Si es necesario, utilizar diluyentes estériles apropiados : agua peptona sal (o Diluyente de Máxima Recuperación) (método ISO 6887), tampón fosfato de Butterfield (tampón fosfato IDF, KH$_2$PO$_4$ a 0.0425g/l , ajustar pH a 7.2), agua peptonada al 0.1%, agua peptonada tampónada (método ISO 6579), solución salina (0.85 - 0.90%), caldo letheen sin bisulfito, o agua destilada.

6. Mezclar u homogeneizar la muestra según el procedimiento habitual.

   **Ajustar el pH de la muestra diluida entre 6.5 y 7.5:**
   - para productos ácidos, usar NaOH 1N,
   - para productos alcalinos, usar HCl 1N.

   *No usar tampones que contengan citrato, bisulfito o tiosulfato, ya que pueden inhibir el crecimiento.*

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### Inoculación

7. Colocar la placa Petrifilm en una superficie **plana.** Levantar el film superior. Con una pipeta colocada de forma **perpendicular** a la placa Petrifilm, colocar 5 ml. de la muestra en el centro del film inferior.

8. Bajar el film superior **con cuidado** evitando introducir burbujas de aire. No dejarlo caer.

9. Colocar el **aplicador para Alta Sensibilidad** en el film superior sobre el inóculo. Distribuir la muestra ejerciendo una **ligera presión** sobre el mango del aplicador. No girar ni deslizar el aplicador. Levantar el aplicador. Esperar de 2 a 5 minutos a que solidifique el gel.
**Interpretación**

11. Las placas Petrifilm pueden leerse con un contador de colonias standard u otra lente de aumento iluminada. Para leer los resultados, consultar la Guía de Interpretación.

12. Las colonias pueden aislarse para una posterior identificación. Levantar el film superior y seleccionar la colonia del gel.

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**Incubación**

10. Incubar las placas cara arriba en pilas de hasta 10 placas. El tiempo de incubación y la temperatura varía según el método*.

*Métodos más usuales utilizados en Europa
• Método validado AFNOR 3M 01/7-03/99 : incubar 24h ± 2h a 30°C ± 1°C, ó 35°C ± 1°C ó 37°C ± 1°C, para coliformes totales.
• incubar 24h ± 2h a 44°C ± 1°C, para coliformes termotolerantes
Para esta alta temperatura, es necesario una humidificación del incubador.

*Métodos más usuales utilizados en Estados Unidos
• incubar 24h ± 2h a 32°C ± 1°C (productos lácteos)
• incubar 24h ± 2h a 35°C ± 1°C (todos los alimentos, excepto productos lácteos)
*Ver el folleto del producto.

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**Diluciones**

Diluciones recomendadas
• Para yogurts, mantequilla y productos lácteos deshidratados, se recomienda una dilución 1 : 10. Esto da una sensibilidad de 2 coliformes por gramo.
• Para nata, helados, leche con chocolate y nata fermentada, se recomienda una dilución 1 : 5. Esto da una sensibilidad de 1 coliforme por gramo.
• Leche cruda, pasterizada entera y descremada, se puede sembrar directamente.

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3M España, S.A.
Juan Ignacio Luca de Tena, 19-25
Madrid 28027, España
Tel. : 34-1-321-60-00
Fax : 34-1-321-60-02
A las 14 horas de incubación

Recuento de colonias de coliformes (8-24 h)

Pueden empezar a aparecer colonias rojas con o sin gas ya a las 8 h y continuar creciendo en el transcurso de la incubación.

- Interpretación al comparar con los métodos AOAC/BAM
  Contar las colonias rojas asociadas a gas como coliformes confirmados.

- Interpretación al comparar con el ISO 4831(NMP)
  Contar las colonias rojas asociadas a gas como coloniformes. Resultados finales a las 24 ± 2 h (validación AFNOR), excepto para carne de cerdo procesada.

- Interpretación al comparar con el ISO 4832(VRBL)
  Contar las colonias rojas con o sin gas como coliformes. Resultados finales a las 24 ± 2 h (validación AFNOR).
La lectura temprana del crecimiento Petrifilm Serie 2000 para Recuento (medida por la producción de ácido de bacterias, su estado metabólico)

**Recuento de Zonas Ácidas (6-14 h)**

Observar el cambio del gel en las figuras 3 a 10. Como los coliformes producen ácido, el color del gel cambia de rojo-anaranjado a naranja-amarillento.

Altas concentraciones de coliformes (>1000 colonias / placa) pueden causar que toda el área de crecimiento se vuelva amarilla tras 4 horas de incubación. Ver Figura 4. Cuando ésto ocurra, se debe diluir más la muestra para obtener un recuento más exacto.

Algunos coliformes producen gran cantidad de ácido. En este caso, se podría dar una fusión de las zonas ácidas sólo con 20 colonias por placa. Se pueden hacer estimaciones en placas que contengan más de 50 zonas ácidas claras.

El área circular de crecimiento en una placa Petrifilm RCC es de aproximadamente 20 cm². Se pueden hacer estimaciones contando el número de zonas ácidas en uno o más cuadrados representativos, determinando el promedio por cuadrado y multiplicando por 20.

Hay 6 zonas ácidas en el cuadrado marcado en la Figura 5. Empezarán a aparecer colonias rojas en las zonas ácidas al continuar creciendo los coliformes. Ver Figura 6.
bacteriano en las placas
Rápido de Coliformes y gas) depende del tipo y su concentración.

Recuento de colonias y gas (8-24 h)
Las Figuras 7 y 8 muestran los resultados para la misma concentración de diferentes organismos incubados durante el mismo tiempo. Aparecen claramente visibles colonias rojas con zonas ácidas en ambas placas. Los organismos de la Figura 8 parece que fermentan la lactosa para producir gas más fácilmente que los de la Figura 7.

Contar las colonias con o sin gas, según el método seguido. Una colonia está asociada a una(s) burbuja(s) si ésta está separada como máximo un diámetro de una colonia o en forma de un anillo alrededor de la misma.
Ver Círculos 1 y 2 respectivamente de la Figura 7.

*La Figura 9 es otro ejemplo de recuento de colonias con o sin burbujas de gas. El recuento depende del método seguido.
• Comparado con los métodos AOAC/BAM, las colonias confirmadas de coliformes con gas = 72.
• Comparado con la ISO 4831, los coliformes son colonias con gas = 72.
• Comparado con la ISO 4832, los coliformes son colonias con y sin gas = 128.

Cuando el número de colonias es más de 150 por placa, se debe estimar el recuento. No contar colonias que aparecen en el límite del círculo, ya que se hallan fuera de la influencia selectiva del medio. Ver Figuras 7 - 10.

Recuento de coliformes estimado = 240

(Ver texto para el recuento de coliformes*)

Recuento de colonias = 64

Recuento de colonias = 164
Las placas Petrifilm RCC con un número de colonias Demasiado Numeroso Para contar (TNTC) tienen una o más de las siguientes características: cambio del color del gel de rojo-anaranjado a naranja-amarillento, muchas colonias pequeñas, muchas burbujas de gas.

La placa Petrifilm RCC de la Figura 12 tiene dos características que indican colonias TNTC: cambio del color del gel y muchas colonias pequeñas.

Recuento de coliformes = TNTC (recuento real > 10⁹)

En la Figura 13, el recuento es tan alto que no aparecen colonias individuales. Un cambio del color del gel a amarillo y muchas burbujas de gas indican colonias TNTC.

La Figura 14 muestra una placa Petrifilm RCC con un alto número de colonias Gram-negativas no-coliformes. Cuando un alto número de organismos que no fermentan la lactosa están presentes, el gel puede aparecer de color rojo oscuro.

Recuento de coliformes = 0
pH: La mayoría de bacterias muestran un crecimiento óptimo a pH cercano a 7.0. Las diluciones de productos de pH bajo requieren un reajuste del pH a 6.5 - 7.5 antes de inocular las placas Petrifilm.

Las Figuras 15 y 16 muestran ejemplos de yogurt fresco sembrado tras el reajuste de pH. Los inhibidores del medio evitan que crezca el cultivo starter Gram positivo, pero el ácido producido por el cultivo starter puede cambiar el color base del gel de rojo-anaranjado a naranja-amarillento, simulando un resultado TNTC temprano. Realizar placas control con un cultivo de yogurt fresco durante la incubación para comprobar que continúa el crecimiento de coliformes TNTC.

Producto: Las partículas alimenticias a menudo son de forma irregular y no se hallan asociadas a burbujas de gas

La Figura 17 es una lectura temprana de una dilución de pimienta negra. El Círculo 1 muestra una zona ácida alrededor de una partícula alimenticia roja y de forma irregular. Algunos alimentos pueden contener partículas ácidas que reaccionen con el indicador de pH. El Círculo 2 muestra una burbuja próxima a una partícula alimenticia roja, de forma irregular, pero no una zona ácida. Tampoco este caso debe ser contado como una colonia.

En la Figura 18 se muestra una dilución de chocolate. Durante la incubación, las zonas de ácido asociadas con colonias continuarán su extensión. Las burbujas de gas asociadas a colonias son otros criterios que ayudarán en la identificación de los coliformes. Las burbujas de gas pueden perfilar la colonia, como se muestra en el Círculo. El recuento con o sin gas depende del método seguido.
Instrucciones de uso

Almacenamiento

1. Conservar las bolsas cerradas a ≤8°C. Usar antes de la fecha de caducidad impresa en la bolsa.
2. Para cerrar las bolsas que se están utilizando, doblar los extremos y cerrarlos con celo.
3. Mantener las bolsas una vez cerradas a ≤25°C, a HR <50%. No refrigerar las bolsas abiertas. Usar las placas Petrifilm en un mes desde su apertura.

Preparación

4. La leche con alto y bajo contenido de grasa puede inocularse directamente. Para otros productos alimenticios o lácteos, diluir la muestra al menos al 1:10. Pesar o pipetear el producto alimenticio en un contenedor estéril adecuado, como una bolsa tipo Stomacher, frasco de dilución, bolsa Whirl-Pak®, o cualquier otro contenedor estéril.
5. Usar diluyentes estériles apropiados: peptona sal (método ISO 6887) (o Diluyente de Máxima Recuperación), tampón fosfato de Butterfield (tampón fosfato IDF, KH₂PO₄ a 0.0425g/l, ajustar pH a 7,2), agua peptonada al 0.1%, solución salina (0.85 - 0.90%) o agua destilada.
6. Mezclar u homogeneizar la muestra mediante los métodos habituales.

No usar tampones que contengan citrato, bisulfito o tiosulfato.
Ajustar el pH de la muestra diluida entre 6.5 y 7.5: para productos ácidos, usar NaOH 1N, para productos alcalinos, usar HCl 1N.

Inoculación

7. Colocar la placa Petrifilm en una superficie plana. Levantar el film superior.
8. Con una pipeta colocada de forma perpendicular a la placa Petrifilm, colocar 1 ml. de la muestra en el centro del film inferior.
Con la cara lisa hacia abajo, colocar el aplicador en el film superior sobre el inóculo. 

Con cuidado, ejercer una presión sobre el aplicador para repartir el inóculo sobre el área circular. No girar ni deslizar el aplicador. 

Levantar el aplicador. Esperar un minuto a que solidifique el gel. 

10 Con la cara lisa hacia abajo, colocar el aplicador en el film superior sobre el inóculo. 
11 Con cuidado, ejercer una presión sobre el aplicador para repartir el inóculo sobre el área circular. No girar ni deslizar el aplicador. 
12 Levantar el aplicador. Esperar un minuto a que solidifique el gel. 

Incubación 

Incubar las placas cara arriba en pilas de hasta 20 placas a temperatura de 35°C ± 1°C durante 24h ± 2h, examinando las placas a intervalos determinados, según la información que se desee obtener (ver el folleto del producto). 

*Ver el folleto del producto para la excepción que hace la AFNOR en la temperatura de incubación para la carne de cerdo procesada. 

Interpretación 

Leer las placas Petrifilm usando una luz indirecta para una detección temprana. Las placas Petrifilm pueden leerse con un contador de colonias standard u otra lente de aumento. Para leer los resultados, consultar la Guía de Interpretación. 

Las colonias pueden aislar para una posterior identificación. Levantar el film superior y seleccionar la colonia del gel. 

13 Incubar las placas cara arriba en pilas de hasta 20 placas a temperatura de 35°C ± 1°C durante 24h ± 2h, examinando las placas a intervalos determinados, según la información que se desee obtener (ver el folleto del producto). 

14 Leer las placas Petrifilm usando una luz indirecta para una detección temprana. Las placas Petrifilm pueden leerse con un contador de colonias standard u otra lente de aumento. Para leer los resultados, consultar la Guía de Interpretación. 

15 Las colonias pueden aislar para una posterior identificación. Levantar el film superior y seleccionar la colonia del gel. 

Comentarios adicionales 

La interpretación de las colonias de coliformes en el Petrifilm Serie 2000 para Recuento Rápido de Coliformes varía según el método utilizado; ver el folleto del producto.
• **3M™ Petrifilm™**
  Recuento de Aerobios
  - Réf.: 06400 / 100 unidades
  - Réf.: 06406 / 1000 unidades

• **3M™ Petrifilm™**
  Recuento de Enterobacteriaceae
  - Réf.: 06420 / 50 unidades
  - Réf.: 06421 / 1000 unidades

• **3M™ Petrifilm™**
  Recuento de Coliformes
  - Réf.: 06410 / 50 unidades
  - Réf.: 06416 / 1000 unidades

• **3M™ Petrifilm™**
  Recuento de Coliformes Alta Sensibilidad
  - Réf.: 06405 / 50 unidades
  - Réf.: 06415 / 500 unidades

• **3M™ Petrifilm™**
  Recuento de E.coli y Coliformes
  - Réf.: 06404 / 50 unidades
  - Réf.: 06414 / 500 unidades

• **3M™ Petrifilm™**
  Recuento de Levaduras y Mohos
  - Réf.: 06407 / 100 unidades
  - Réf.: 06417 / 1000 unidades

• **3M™ Petrifilm™** Serie 2000
  Recuento Rápido de Coliformes
  - Réf.: 06402 / 50 unidades
  - Réf.: 06412 / 500 unidades

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Laboratoires 3M Santé
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Esta guía le familiarizará con los resultados obtenidos con las placas Petrifilm™ Select E. coli (SEC). Si desea más información, contacte con el representante habitual de Productos de Microbiología de 3M.

**Petrifilm™**
Placas para recuento selectivo de E. coli

Esta guía le familiarizará con los resultados obtenidos con las placas Petrifilm™ Select E. coli (SEC). Si desea más información, contacte con el representante habitual de Productos de Microbiología de 3M.

**Recuento de E. coli = 97**
Las placas Petrifilm Select E. coli permiten la detección específica de E. coli. Aproximadamente un 97% de las cepas de E. coli producen β-glucuronidasa, que reacciona con un colorante indicador presente en la placa produciendo colonias con una gama de colores de verde oscuro a azul verdoso.

**Las placas Petrifilm SEC no detectarán E. coli O 157, porque la mayoría de las cepas E. coli O 157 no producen glucuronidasa**

**Recuento de E. coli = 0**
Es difícil ver colonias distintas de E. coli, porque o son incoloras o tienen un color gris claro- beige.
Placas 3M™ Petrifilm™ para recuento selectivo de E. coli

Intervalo de recuento:
El intervalo de recuento de las placas Petrifilm Select E. coli es de 15 a 150 colonias. Un número de colonias mayor de 150 puede estimarse o considerarse demasiado alto para el recuento (TNCT). Para conseguir un recuento preciso, diluir adicionalmente la muestra.

Recuento de E. coli = 56
No contar las colonias de la zona porosa, porque están exentas de la influencia selectiva del medio. Ver circulo 1.

Recuento de E. coli estimado ≈ 740
Cuando el número de colonias es mayor de 150, pueden realizarse estimaciones. Contar el número de colonias de uno o más cuadrados representativos y multiplicar el número medio por 20 para obtener el recuento estimado por placa.

Recuento de E. coli = TNTC
Cuando están presentes en gran número, las colonias de E. coli pueden ser pequeñas y poco definidas.

Recuento de E. coli = TNTC
Una concentración elevada de E. coli hará que todo el área de crecimiento tome un color azul-verdoso.
Placas 3M™ Petrifilm™ para recuento selectivo de E. coli

Interferencias debidas a alimentos:
Las placas Petrifilm Select E. coli se han evaluado usando muestras de muchos, pero no de todos los alimentos. Los alimentos ensayados incluyen ciertas carnes frescas y congeladas, verduras y mariscos; comidas preparadas congeladas; y productos lácteos frescos, fermentados y deshidratados. En un número limitado de casos, tales como cuando el alimento es hígado, éste puede interferir con la enumeración. Para reducir tal interferencia del alimento, diluir adicionalmente la muestra.

Recuento de E. coli = 42
Las colonias de E. coli pueden distinguirse fácilmente de las partículas de alimento, ya que éstas a menudo tienen formas irregulares y presentan tamaños y colores variables. El círculo 1 muestra partículas de nuez.

Recuento de E. coli = 21
Algunos alimentos oscuros pueden producir un fondo coloreado que dificulta la diferenciación de las colonias de E. coli. Las diluciones adicionales aclararán el color de fondo facilitando el recuento de las colonias de E. coli. La figura 8 muestra cacao en polvo diluido 1:50.

El hígado crudo contiene β-glucuronidasa, que produce un fondo azul-verdoso del área de crecimiento de las placas que dificulta la diferenciación de las colonias de E. coli. Una dilución adicional aclarará el color de fondo facilitando el recuento de las colonias de E. coli y ayudará a distinguir la interferencia de los alimentos en las placas TNTC que tienen colonias confluentes (véase la figura 6). Se pueden producir burbujas por una inoculación incorrecta de la placa o por quedar aire atrapado en la muestra. Véase el círculo 1.
Recuento de *E. coli* = 92
Pueden aparecer colonias de color verde pálido cuando las células *E. coli* son productores débiles de glucuronidasa o cuando existe una interacción con alimentos que contienen mucho ácido o mucho azúcar.
La Figura 10 muestra un producto lácteo fermentado muy ácido.

Recuento de *E. coli* = 75
Con algunos alimentos puede aparecer una variabilidad de color pardo-verdoso.
La Figura 11 ilustra una muestra de riñón.

Recuento de *E. coli* = 10
Las colonias de *E. coli* pueden tener asociadas burbujas de gas dependiendo de la cepa de *E. coli* y del alimento.
Contar todas las colonias, tengan gas o no.

Recuento de *E. coli* = 25
Pueden aparecer colonias difuminadas. Ver circulo 1.
Para minimizar la producción de colonias difuminadas, presionar *suavemente* el centro del esparcidor y extender inmediatamente después de la inoculación.
3M™ Petrifilm™ Placas para recuento selectivo de E. coli

Advertencias para el uso

Almacenamiento

1. Conservar los envases no abiertos a ≤8ºC. Usar antes de la fecha de caducidad indicada en el envase. En zonas de humedad alta donde la condensación puede ser un problema es mejor dejar que los envases alcancen la temperatura ambiental antes de abrirlos.

2. Para cerrar un envase abierto, doblar el extremo y fijar con cinta adhesiva.

3. Almacenar los envases que se han cerrado en un lugar frío y seco durante no más de un mes. No refrigerar los envases abiertos. Conservar los envases que se han cerrado en un congelador si la temperatura del laboratorio excede de 25ºC y/o el laboratorio está localizado en un área en el que la humedad frecuentemente excede del 50%.

Preparación

4. Pesar o pipetear el producto alimenticio en un recipiente estéril apropiado tal como una bolsa tipo Stomacher, un frasco de dilución o una bolsa Whirl-Pak®.

5. Añadir la cantidad apropiada de uno de los siguientes diluyentes estériles: agua de peptona tamponada (ISO 6887), diluyente de sal de peptona (ISO 6887), agua de peptona al 0,1%, K₂HPO₄ (IDF 122B), tampón fosfato de Butterfield (tampón fosfato IDF 122B, K₂HPO₄ a 0,0425 g/l, ajustado a pH 7,2), caldo de cultivo letheen sin bisulfito, solución de Ringer diluida hasta un cuarto de la concentración normal (IDF 122B), solución salina (0,85-0,90%) o agua destilada.

   Ajustar el pH de la muestra diluida entre 6,5 y 7,5:
   • para productos ácidos, usar NaOH. 1 N,
   • para productos alcalinos, usar HCl 1 N.

No usar tampones que contengan citrato, bisulfito o tiosulfato; ya que pueden inhibir el crecimiento.

Inoculación

7. Colocar la placa Petrifilm sobre una superficie plana. Levantar la película superior.

8. Con la pipeta perpendicular a la placa Petrifilm, poner 1 ml de muestra en el centro de la película inferior.

9. Bajar cuidadosamente la película superior para evitar la acumulación de burbujas de aire. No hay que dejarla caer.
Con la cara lisa hacia abajo, poner el aplicador en la película superior sobre el inóculo.

10

Aplicar presión suavemente sobre el aplicador para distribuir el inoculo en un área circular. No girar ni deslizar el aplicador.

11

Levantar el aplicador. Esperar al menos un minuto para que se forme el gel.

12

Incubación

Incubar las placas con el lado transparente hacia arriba, en pilas de hasta 20 unidades. Incubar durante 24 h ± 2 h a 42ºC ± 1ºC o a 44ºC ± 1ºC. Puede ser necesario humidificar la estufa para minimizar la pérdida de humedad durante la incubación.

13

Interpretación

Las placas Petrifilm pueden leerse con un contador de colonias convencional u otra lente de aumento. Véase la Guía de Interpretación para leer los resultados.

14

Es posible aislar las colonias para realizar identificaciones adicionales. Levantar la película superior y extraer la colonia del gel. Proceder según métodos convencionales.

15

Referencia a documentos

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<th>Versión</th>
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The Petrifilm Staph Express count system consists of a Petrifilm Staph Express count plate and a Petrifilm Staph Express disk.

The Petrifilm Staph Express count plate is a sample-ready culture medium system. The chromogenic, modified Baird-Parker medium in the count plate is selective and differential for *Staphylococcus aureus*. *Staphylococcus aureus* appears as red-violet colonies on the count plate (figure 1). Colonies other than red-violet colonies may appear on the count plates (figure 5).

The Petrifilm Staph Express disk is designed for the detection of desoxyribonuclease (DNase) reactions specific for *Staphylococcus aureus* isolated on the Petrifilm Staph Express count plate; it contains toluidine blue-O that facilitates the visualization of the DNase reactions (figure 2). The Petrifilm Staph Express disk must be used whenever colonies other than red-violet are present on the count plate.

1. Figure 1 shows red-violet colonies of *S. aureus*. *S. aureus* colonies may vary in size.

2. Figure 2 shows pink zones which form when DNase reaction from *S. aureus*. Pink zones may be various sizes because different *S. aureus* produce DNase at varying rates.
Interpretation of 3M™ Petrifilm™ Staph Express Count System

1. Colony count on Petrifilm Staph Express Count Plates
After 24 hours incubation of the count plates, if only red-violet colonies appear, proceed to numeration. To enumerate *Staphylococcus aureus*, count all red-violet colonies. Use an illuminated magnifier so that the colonies are easier to see.

S. aureus count = 20
Figure 4 shows a Petrifilm Staph Express count plate with only red-violet colonies. Count all red-violet colonies regardless of size as *S. aureus*. Also visible on this count plate are irregularly shaped food particles.

2. DNase pink zones count on Petrifilm Staph Express count system
Whenever colonies other than red-violet are seen on the count plate, use a test Petrifilm Staph Express disk to count *Staphylococcus aureus*. Figures 5 and 6 show the same Petrifilm Staph Express count system before and after using the disk.

S. aureus count = 33
Figure 6 shows 33 pink zones produced by the same number of *S. aureus* colonies. Count all pink zones as *S. aureus*. The arrows in the figure show gel splitting. Gel splitting does not affect the performance.
Sample Preparation

1. **Store** unopened packages of count plates and disks at ≤ 8°C (≤ 46°F). Use before expiration date on package. Prior to use, it is best to allow unopened pouches of count plates to come to room temperature before opening to avoid condensation.

2. **To seal opened package**, fold end over and secure with tape or clip.

3. **Count plates**: Store resealed packages in a cool dry place (≤ 25°C) for no longer than one month. Do not refrigerate opened packages. Store them in a freezer if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in an area where the humidity frequently exceeds 50%. **Disks**: Store resealed packages in a freezer (≤ -15°C) for no longer than six months. Do not store opened packages of disks at room temperature.

**Adjust pH of the diluted sample to between 6 and 8:**
- for acid products, use NaOH 1N,
- for alkaline products, use HCl 1N.

4. **Prepare** a 1:10 or greater dilution. Weigh or pipette food product into an appropriate sterile container such as stomacher bag, dilution bottle, or other sterile container.

5. Add appropriate quantity of one of the following sterile diluents recommended as diluents for general use (ISO 6887 and ISO 8261/IDF 122), such as peptone salt diluent and buffered peptone water. Other diluents may also be used such as, for example, bisulfite-free letheen broth.

6. Blend or homogenize sample as per current procedure.

Inoculation

7. Place Petrifilm plate on level surface. Lift top film. With pipette perpendicular to Petrifilm plate, place 1mL of sample onto center of bottom film.

8. **Carefully** roll top film down to avoid trapping air bubbles. Do not let top film drop.

9. Gently apply pressure to the spreader to distribute inoculum over circular area before gel forms. Do not twist or slide the spreader. Lift spreader. Wait at least one minute for gel to solidify.

**Note**: Spread the sample on each individual count plate before inoculating the next. This is important as the gel in the Petrifilm Staph Express count plate forms quickly.
Incubate count plates with clear side up in stacks of up to 20. Incubate for 24 ± 2h at 37°C ± 1°C.

If no colonies are present after 24 ± 2 hours of incubation, the count is zero and the test is complete.

If no colony other than red-violet ones are visible, count them as S. aureus. The test is complete. Count plates can be counted with a standard colony counter or other illuminated magnifier. Refer to the Interpretation Guide section when reading results.

Remove a disk from its individual package by grasping the tab. Lift the top film of the Petrifilm count plate and place the disk in the well of the count plate. Lower the top film.

Apply pressure to the disk area, including the edges of the disk, by sliding a finger firmly across the top film. This will ensure uniform contact of the disk with the gel and will eliminate any air bubbles.

Incubate count plates with inserted disks in stacks of no more than 20 count plates for 3 hours at 37°C ± 1°C.

Count all pink zones whether or not colony is present as S. aureus. Refer to the Interpretation Guide section when reading results.

Colonies may be isolated for further identification. Lift top film and pick the colony from the gel. Test using standard procedures.

Disk Use

If any colony colours besides red-violet are present, use a Petrifilm Staph Express disk (see 13-16).
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.
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: 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 cm².

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.

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.
**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.

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.

   Do not use enrichment broth on this plate.

**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.
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.

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.

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.

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.

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.

Optional

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

\[
\text{CFU/area} = \frac{\text{Number of colonies} \times (\text{mL hydration fluid} + \text{mL BPW})}{3 \text{ mL}} \div \text{area sampled}
\]

Or

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

Environmental quantitative sampling is consistent with the following references:

Quantitative Interpretation

Example: Sponge Contact Method

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

A. Total number of mL of BPW + hydration fluid:
   10 + 5 = 15  

B. Number of mL plated:
   3

C. Divide line A by line B:
   5

D. Number of colonies counted:
   10

E. Multiply line C by line D:
   50

F. Area sampled:
   50 cm²

G. Divide line E by line F:
   1 CFU/cm²

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  

B. Number of mL plated:
   3

C. Divide line A by line B:
   1

D. Number of colonies counted:
   50

E. Multiply line C by line D:
   50

F. Area sampled:
   50 cm²

G. Divide line E by line F:
   1 CFU/cm²
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
Processors of high acid/low pH or vacuum-packed products, such as salad dressings, tomato-based products and processed meats, need to continually monitor for the presence of lactic acid bacteria. Lactic acid bacteria are spoilage organisms known to shorten product shelf life and degrade product quality.

Following a simple procedure, you’ll find 3M™ Petrifilm™ Aerobic Count Plates can be an efficient, cost-effective method for detecting lactic acid bacteria in no more than 48 hours.

Because of its unique construction, Petrifilm Aerobic Count plates can differentiate important gas-producing organisms (heterofermenters) from non-gas-producing organisms (homofermenters). In fact, Petrifilm plates are more sensitive than the MRS tube method at detecting these destructive gas-producing organisms.

By using labor-saving Petrifilm plates, you’ll have more time to monitor critical control points more frequently. The end result is better process control and a higher quality product.
Lactic Acid Bacteria Detection.

**Fast, accurate testing.** Only four simple steps are required.
1. Prepare sample using standard methods with MRS broth in final dilution step.
2. Inoculate and spread Petrifilm Aerobic Count plate with one mL of sample.
3. Incubate **anaerobically** at the appropriate temperature.
4. Count the colonies.

Because Petrifilm plates are consistent and easy-to-use, there’s less chance for error when compared to other methods.

A built-in grid facilitates counting colonies, giving you fast, precise and consistent results.

3M offers the 3M™ Redigel™ MRS Test as an additional option for lactic acid bacteria testing.

**3M Reliability.** All Petrifilm plates are manufactured at an ISO 9002-certified site. Strict quality control procedures help reduce media variations. They are backed by 3M’s commitment to quality products, customer service, and technical support. In addition, most Petrifilm plate methods have been collaboratively tested and are included in the **Official Methods of Analysis** of AOAC INTERNATIONAL.

There’s a full line of Petrifilm plates to monitor conditions for quality: coliform count, rapid coliform count, high-sensitivity coliform count, aerobic count, E. coli/coliform count, yeast and mold count, Enterobacteriaceae count and rapid S. aureus count.

For detailed CAUTIONS, LIMITED WARRANTY and LIMITED REMEDIES, STORAGE AND DISPOSAL information, and INSTRUCTIONS FOR USE see Product’s package insert.

---

### Ordering Information

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<thead>
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<th>Product</th>
<th>Application</th>
<th>Catalog No.</th>
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<td>For lactic acid bacteria</td>
<td>6400</td>
<td>100 plates</td>
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<tr>
<td>plates (AC)</td>
<td>identification</td>
<td>6406</td>
<td>1000 plates</td>
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</tbody>
</table>

To order Petrifilm products in the U.S., call **1-800-328-1671**. Latin America/Africa and Asia Pacific regions, call **651-733-7562**.

---

**Microbiology Products**

3M Center, Building 275-5W-05  
St. Paul, MN 55144-1000 USA  
1 800 228-3957  
www.3M.com/microbiology  
E-mail: microbiology@3M.com

**3M Canada Inc.**

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1 800 364-3577

**3M Europe**

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France  
33 1 30 31-8571

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10% Post-consumer waste paper

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70-2009-3271-6 (90.8) DPI
**Interpretation Guide**

**Lactic Acid Bacteria Count**

The Petrifilm Aerobic Count plate is used in combination with an MRS broth diluent and anaerobic incubation to enumerate lactic acid bacteria. For more information, contact the official 3M Microbiology Products representative nearest you.

---

**Sample Preparation**

1. Prepare at least a 1:10 dilution of the sample if the product is a broth or saline solution. For solid or high viscosity foods, dilute the sample in a 1:10 ratio. Multiple microbial tests will be run from a single 1:10 dilution, a single 1:10 dilution, or a multiple dilution step.

2. Add appropriate quantity of MRS broth diluent to each tube of sample. Add 5 mL of sample/volume broth to the broth diluent. If multiple microbial tests will be run from a single 1:10 dilution, a Multiple Testing Pipette Procedure may be more convenient (see other side).

3. Inoculate Petrifilm plates with 1.0 mL of sample for the MRS broth diluent. Place entire jar into the incubator. Petrifilm plates may be incubated in the same jar if each stack is separated by a rigid divider.

4. Incubate plates anaerobically. Place Petrifilm plates in the GasPak jar in stacks of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same gas jar. Use for Growing Lactic Acid Bacteria

---

**Interpretation**

6. Petrifilm plates can be counted on a standard colony counter or other counting apparatus. For Petrifilm colonies, multiply the count by the dilution factor to determine the number of colonies per mL. Refer to the Interpretation Guide Section.

---

**Organisms**

<table>
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<tr>
<td>Lactobacillus delbrueckii subsp. lactis</td>
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<tr>
<td>Lactobacillus delbrueckii</td>
<td>+++</td>
</tr>
<tr>
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<td>++</td>
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<td>Lactobacillus acidurici</td>
<td>++</td>
</tr>
<tr>
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<td>-</td>
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<tr>
<td>Lactobacillus delbrueckii</td>
<td>++</td>
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<td>Lactobacillus brevis</td>
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<td>Lactobacillus casei</td>
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</tr>
<tr>
<td>Lactobacillus bulgaris</td>
<td>+</td>
</tr>
<tr>
<td>Lactobacillus fermentum</td>
<td>++</td>
</tr>
</tbody>
</table>

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**Additional Comments**

- For technical assistance, call 1-800-328-6553.
- To order Petrifilm plates in the U.S., call 1-800-333-6471.
- Latin America / Asia And Pacific region, call +44-734-7025.

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**Use for Growing Lactic Acid Bacteria**

This guide familiarizes you with results on 3M™ Petrifilm Aerobic Count Plates when used to grow lactic acid bacteria. For more information, contact the official 3M Microbiology Products Representative nearest you.
Figure 3 shows a Petrifilm Aerobic Count plate inoculated with an MRS broth diluent. This is referred to as an “MRS diluent control.” The MRS diluent and anaerobic incubation cause a slightly shaded growth area with a pale ring.

The preferable counting range is 25–250 colonies. Count all colonies regardless of size or color intensity.

When you look closely, you can see small pinpoint colonies both in the center and on the edge of the growth area. Record this as a TNTC.

When colonies number more than 250, as shown in figure 5, estimate the count. Determine the average number of colonies in one square (1 cm²) and multiply it by 20 to obtain the total count per plate. The inoculated area on a Petrifilm Aerobic Count plate is approximately 20 cm².

When colonies are too numerous to count (TNTC), the entire growth area may turn pink, as shown in figure 6. Compare incubated plates to an MRS diluent control because change in background color may be minimal (see figure 3 for MRS control). Further dilution of the sample may be necessary.

If it is more convenient to use an existing 1:10 dilution made with a standard diluent, instead of preparing a new 1:10 dilution specifically for lactic evaluation, the following multiple testing procedure can be used:

**Sample Preparation**

1. Prepare MRS broth to a concentration of 2 times (2X) the suggested manufacturer’s quantity.
2. Prepare a 1:10 dilution with standard diluent (1% MRS, 11 g/99 mLs). Plate up to 8 mLs for other microbial tests.
3. Add concentrated MRS broth (4x) solution to existing 1:10 dilution. This will result in half-strength MRS concentration.

   - If the dilution involves adding 11 grams of product to 99 mLs diluent, plate other microbial tests and then add 18 mLs of the concentrated (4x) MRS solution. Multiply final plate count by 11 for count/gram.
   - If the dilution involves adding 25 grams of product to 225 mLs diluent, plate other microbial tests and then add 41 mLs of the concentrated (4x) MRS solution. Multiply final plate count by 11 for count/gram.

**Multiple Testing Procedures**

1. Prepare MRS broth to a concentration of 2 times (2X) the suggested manufacturer’s quantity.
2. Prepare a 1:10 dilution with standard diluent (1% MRS, 11 g/99 mLs). Plate up to 8 mLs for other microbial tests.
3. Add concentrated MRS broth (4x) solution to existing 1:10 dilution. This will result in half-strength MRS concentration.

   - If the dilution involves adding 11 grams of product to 99 mLs diluent, plate other microbial tests and then add 18 mLs of the concentrated (4x) MRS solution. Multiply final plate count by 11 for count/gram.
   - If the dilution involves adding 25 grams of product to 225 mLs diluent, plate other microbial tests and then add 41 mLs of the concentrated (4x) MRS solution. Multiply final plate count by 11 for count/gram.
Count = 0

Figure 3 shows a Petrifilm Aerobic Count plate inoculated with an MRS broth diluent. This is referred to as an “MRS diluent control.” The MRS diluent and anaerobic incubation cause a slightly shaded growth area with a pale ring. Count = 60

The preferable counting range is 25–250 colonies. Count all colonies regardless of size or color intensity. Count = TNTC (Estimated count = 10^6)

When you look closely, you can see small pinpoint colonies both in the center and on the edge of the growth area. Record this as a TNTC.

Count = TNTC

The Petrifilm plate in figure 9 is an example of a TNTC plate. Both homofermentative (non-gas-producing) colonies (see circle 1) and heterofermentative (gas-producing) colonies (see circle 2) are present. Count = 52

Artifact bubbles may result from improper inoculation of the Petrifilm plate. They are irregularly shaped and not associated with a colony. Count = TNTC (Estimated count = 10^8)

With very high counts, small pinpoint colonies may surround the circular growth area. Record this as a TNTC.

Estimated Count = 440

When colonies number more than 250, as shown in figure 5, estimate the count. Determine the average number of colonies in one square (1 cm²) and multiply it by 20 to obtain the total count per plate. The inoculated area on a Petrifilm Aerobic Count plate is approximately 20 cm². Count = TNTC (Estimated count = 10^6)

When colonies are too numerous to count (TNTC), the entire growth area may turn pink, as shown in figure 6. Compare incubated plates to an MRS diluent control because change in background color may be minimal (see figure 3 for MRS control). Further dilution of the sample may be necessary.

Multiple Testing Procedures
If it is more convenient to use an existing 1:10 dilution made with a standard diluent, instead of preparing a new 1:10 dilution specifically for lactic evaluation, the following multiple testing procedure can be used:

MRS 2X Concentration Procedure

Sample Preparation

1. Prepare MRS broth to a concentration of 2 times (2X) the suggested manufacturer’s quantity and sterile. For example, if MRS is prepared by adding 55 grams to 1 liter, instead add 110 grams of MRS to 1 liter.

2. Prepare a 1:10 dilution with standard diluent. Use the 1:10 dilution to plate other microbial tests.

3. Make a 1:2 dilution of your 1:10 dilution by using the 1 mL 3M Electronic Pipettor. Program the pipettor to make a 1:2 dilution. First draw up 0.5 mL of the 1:10 dilution, then draw up 0.5 mL of the 1:2 dilution. This will result in a 1:20 dilution with a concentration of single strength MRS broth.

Inoculation

4. Plate 1 mL of the 1:20 sample dilution (0.5 mL, 1:10 dilution + 0.5 mL, 2X concentration MRS = 1:20 dilution) anaerobically. Place Petrifilm plates in stacks of no more than 20 plates in a single GasPak jar. Each stack should be separated by a rigid divider. Place entire jar into the incubator. Incubate Petrifilm plates at 30°-35°C (86°-95°F) for 48 ± 3 h.

5. The Petrifilm plate in figure 9 is an example of a TNTC plate. Both homofermentative (non-gas-producing) colonies (see circle 1) and heterofermentative (gas-producing) colonies (see circle 2) are present. Further dilution of the sample may be necessary.

Interpretation

6. Petrifilm plates can be counted on a standard colony counter or other magnified light source. Count all colonies, multiply the colony the count by the dilution factor (20), and refer to the Interpretation Guide Section.

MRS 4X Concentration Procedure

1. Prepare MRS broth to a concentration of 4 times (4X) the suggested manufacturer’s quantity.

2. Prepare a 1:10 dilution with standard diluent (11g/99 mL or 25g/225 mL). Plate up to 8 mLs for other microbial tests.

3. Add concentrated MRS broth (4X) solution to existing 1:10 dilution. This will result in half-strength MRS concentration.

• If the dilution involves adding 11 grams of product to 99 mLs diluent, plate other microbial tests and then add 18 mLs of the concentrated (4X) MRS solution. Multiply final plate count by 11 for count/gram.

• If the dilution involves adding 25 grams of product to 225 mLs diluent, plate other microbial tests and then add 41 mLs of the concentrated (4X) MRS solution. Multiply final plate count by 11 for count/gram.

Interpretation

1. Prepare MRS broth to a concentration of 2 times (2X) the suggested manufacturer’s quantity.

2. Prepare a 1:10 dilution with standard diluent (11g/99 mL or 25g/225 mL). Plate up to 8 mLs for other microbial tests.

3. Add concentrated MRS broth (4X) solution to existing 1:10 dilution. This will result in half-strength MRS concentration.

• If the dilution involves adding 11 grams of product to 99 mLs diluent, plate other microbial tests and then add 18 mLs of the concentrated (4X) MRS solution. Multiply final plate count by 11 for count/gram.
**Multiple Testing Procedures**

If it is more convenient to use an existing 1:10 dilution made with a standard diluent, instead of preparing a new 1:10 dilution specifically for lactic evaluation, the following multiple testing procedure can be used:

### MRS 2X Concentration Procedure

#### Sample Preparation

1. Prepare MRS broth to a concentration of 2 times (2X) the suggested manufacturer’s quantity and sterilize. For example, if MRS is prepared by adding 55 grams to 1 liter, instead add 110 grams of MRS to 1 liter.

2. Prepare a 1:10 dilution of your sample with standard diluent. Use the 1:10 dilution to plate other microbial tests.

3. Make a 1:2 dilution of your 1:10 dilution by using the 1 mL 3M Electronic Pipettor. Program the pipettor to make a 1:2 dilution. Then draw up 0.5 mL of the 1:10 dilution, then draw up 0.5 mL of the 2X concentration MRS broth. This will result in a 1:20 dilution with a concentration of single strength MRS broth.

#### Inoculation

1. Plate 1 mL of the 1:20 sample dilution (20 mL, 1:10 dilution + 0.5 mL of 2X concentration MRS = 1:20 dilution)

2. Incubate Petrifilm plates anaerobically (face Petrifilm plates with the clear side up into the GasPak jar and stack up to 20 plates of Petrifilm plates may be incubated in the same jar if stacked using a rigid divider).

3. Place entire jar into the incubator. Incubate plate at 30°-35°C (86°-95°F) for 48 ± 3h.

4. Place Petrifilm plates can be counted on a standard colony counter or other magnified light source. Count all colonies, multiply by the number of colonies per mL. Refer to the Interpretation Guide Section.

#### Interpretation

- **Interpretation Guide**
  - **Counts <70°F**
  - **Counts >70°F**

### MRS 4X Concentration Procedure

1. Prepare MRS broth to a concentration of 4 times (4X) the suggested manufacturer’s quantity.

2. Prepare a 1:10 dilution with standard diluent (11g/99 mL or 25g/225 mL). Plate up to 8 mLs for other microbial tests.

3. Add concentrated MRS broth (4X) solution to existing 1:10 dilution. This will result in half-strength MRS concentration.

4. If the dilution involves adding 11 grams of product to 99 mLs diluent, plate other microbial tests and then add 18 mLs of the concentrated (4X) MRS solution. Multiply final plate count by 11 for count/gram.

5. If the dilution involves adding 25 grams of product to 225 mLs diluent, plate other microbial tests and then add 41 mLs of the concentrated (4X) MRS solution. Multiply final plate count by 11 for count/gram.
**Petrifilm Aerobic Count Plates**

- **Lactic Acid Bacteria Method**
- **Use for Growing Lactic Acid Bacteria**

This guide familiarizes you with results on 3M® Petrifilm Aerobic Count Plates when used to grow lactic acid bacteria. For more information, contact the official 3M Microbiology Products Representative nearest you.

**Interpretation Guide**

To order Petrifilm plates in the USA, call 1-800-332-1671.

To order Petrifilm plates in Canada, call 1-800-328-1671.

For technical assistance, call 1-800-328-6553.

**Differentiates Gas Producers from Non-Gas Producers**

The 3M Petrifilm plate method was compared to the MRS agar method for recovering lactic acid bacteria in 161 naturally contaminated food products. The Petrifilm plate method was more sensitive than the MRS tube method at identifying gas producing and facultative heterofermenters. The following table gives examples of gas production from some of the organisms evaluated.

**Organisms**

- **Obligate heterofermenters:**
  - Lactobacillus fermentum
  - Lactobacillus brevis
  - Lactobacillus plantarum
  - Lactobacillus curvatus
  - Lactobacillus amylovorus
  - Lactobacillus delbrueckii subsp. lactis
  - Lactobacillus delbrueckii subsp. delbrueckii
  - Lactobacillus plantarum subsp. plantarum
  - Lactobacillus casei
  - Lactobacillus gasseri

- **Facultative heterofermenters:**
  - Lactobacillus plantarum subsp. plantarum
  - Lactobacillus casei subsp. rhamnosus
  - Lactobacillus reuteri

- **Obligate homofermenters:**
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus
  - Lactobacillus acidophilus

**Sample Preparation**

1. Prepare at least a 1:10 dilution of each sample. Weight or measure food product into Whirl-Pak® bag, stomacher bag, dilution bottle, or other appropriate sterile container.
2. Add appropriate quantity of MRS broth diluent to each sample. Weight or measure MRS broth diluent to manufacturer's instructions. If multiple inoculants will be used, then single 1:10 dilutions in a Multiplex Testing Procedure may be more convenient (see other side).
3. Inoculate Petrifilm plates with 1.0 mL of sample according to manufacturer's instructions. If multiple inoculants will be used, then single 1:10 dilutions, a single 1:10 dilution, or a higher of food sample. Weigh or measure MRS broth diluent. Prepare according to manufacturer's instructions. If necessary the MRS broth diluent may be more convenient (see other side).
4. Incubate Petrifilm plates with the clear side up into an incubator. Place entire jar into the incubator. Petrifilm plates can be counted on a magnified light source. Count all colonies.
5. Petrifilm plates can be counted on a standard colony counter or other automated colony counter. The greater the number of colonies, multiply the count by the division factor to determine the number of colonies per mL. Refer to the Interpretation Guide Section.

**Count Guide**

- a. MRS is a common medium for detecting lactic acid bacteria in foods.
- b. 8 out of 10 strains were negative.
- c. 30% of the smallest edge may not produce visible gas (see note 2).
- d. Colonies are visible bacteria in color and may or may not be associated with a gas bubble. The colonies in figure 1 are examples of characteristic heterofermentative (non-gas producing) organisms.
The addition of MRS broth to Petrifilm Aerobic Count plates, in combination with anaerobic incubation, enhances the growth of other appropriate sterile container. The unique construction of Petrifilm plates makes it possible to distinguish gas-producing heterofermentative organisms from non-gas-producing homofermenters. Petrifilm™ Aerobic Count plates can be used to enumerate lactic acid bacteria in certain foods. DISPOSAL information, and INSTRUCTIONS FOR USE see Product’s package insert. The Petrifilm Aerobic Count Plate is an alternative to the MRS tube method when used to grow lactic acid bacteria. For more information, contact the official 3M Microbiology Products representative nearest you.

**Sample Preparation**

1. Prepare at least 1:10 dilutions of sample from the sample. Weight of dry substrate is 10% of manufacturer’s instructions. If multiple inoculated test tubes are single 1:10 dilutions, a Multi-Testing Pipette Procedure may be more convenient (see other side).

2. Add appropriate quantity of MRS broth diluent to Petrifilm plates. Place entire jar into the incubator.

3. Incubate plates at 30°-35°C (86°-95°F) for 48 ± 3h. Plates may be incubated in the same GasPak jar in stacks of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same Petrifilm bag. Do not use bags containing chloride, bisulfite, or thiosulfate; they can inhibit growth. Do not use buffers containing citrate, phosphate buffer, 0.0425 g/L of KH2PO4 adjusted to pH 7.2, 0.1% peptone water, peptone salt diluent (ISO method 6887), buffered peptone water (ISO method 6579), saline solution (0.85-10% Post-consumer waste paper 40% Pre-consumer waste paper 1/4 inch of the circle’s edge may not produce visible gas. Heterofermentative colonies within approximately 1 to 2 days, but no gas bubble. The colonies in figure 1 contain both heterofermentative (gas-producing) and homofermentative (non-gas-producing) organisms. The colonies in figure 2 may not be associated with a gas bubble. The colonies in figure 3 are examples of characteristic heterofermentative (non-gas-producing) organisms.

4. Place aseptically into the incubator. Incubate plates at 20°-30°C (68°-86°F) for 48 ± 3h.

5. Inoculate Petrifilm plates with 1.0 mL of sample according to manufacturer’s instructions in package insert.

6. Petrifilm plates can be counted on a standard colony counter or other image analysis systems. Differentiate Gas Producers from Non-Gas Producers: The Petrifilm plate method was compared to the MRS tube method for recovering lactic acid bacteria in 161 naturally contaminated food products. The Petrifilm plate method was more sensitive than the MRS tube method at identifying gas-producing and facultative heterofermenters. The following table gives examples of gas production from some of the organisms evaluated.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Gas Production</th>
<th>Petrifilm Plates</th>
<th>MRS Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obligate heterofermenters:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(These organisms produce gas)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus fermentum</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lactobacillus buchneri</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lactobacillus curvatus</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lactobacillus delbrueckii</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Facultative heterofermenters:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(These organisms may produce gas)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus plantarum (3 strains total)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lactobacillus curvatus (2 strains total)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lactobacillus plantarum (3 strains total)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Obligate homofermenters:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(These organisms do not produce gas)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus defluvii/subsp. boyceii</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus defluvii/subsp. brevis</td>
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<td>-</td>
</tr>
<tr>
<td>Lactobacillus amyloliquefaciens</td>
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<td>Lactobacillus lactis</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Lactobacillus brevis</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus helveticus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Additional Comments**

- For technical assistance, call 1-800-328-6557.
- To order Petrifilm plates in the U.S., call 1-800-228-3957.
- www.3M.com/microbiology
- 1-651-733-7562.
- ©3M 2000

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Lactic Acid Bacteria Count 

The Petrifilm Aerobic Count Plate is an alternative to the MRS tube method when used to grow lactic acid bacteria. For more information, contact the official 3M Microbiology Products representative nearest you.
It is easy to count yeast and mould colonies on Petrifilm Yeast and Mould count plates. An indicator dye stains yeast and mould colonies to provide contrast and facilitate counting.

To differentiate yeast and mould colonies on Petrifilm Yeast and Mould count plates, look for one or more of the following typical characteristics:

**Yeast**
- Small colonies
- Colony has defined edges
- Pink-tan to blue-green in colour
- Colony may appear raised ("3D")
- Usually no focus (dark centre) in middle of colony

The colonies in figure 1 are characteristic examples of yeasts: small, blue-green colonies, with defined edges, and no foci.
(Yeast count = 44)

**Mould**
- Large colonies
- Colony has diffuse edges
- Variable colour (moulds may produce their own pigments)
- Colonies appear flat
- Usually a focus in centre of colony

The colonies in figure 2 are characteristic examples of moulds: large, variable coloured colonies, with diffuse edges, and centre foci.
(Mould count = 27)
The 3M™ Petrifilm™ Yeast and Mould Count Plate in figure 3 contains an easily countable number of mould colonies, (large, green colonies with diffuse edges, and centre foci) and a high number of yeast colonies. The yeast colonies are small, tan colonies with defined edges, and no foci. When colonies number more than 150, estimate the count. **(Yeast count = 480 (estimate); Mould count = 21)**

Determine the average number of colonies in one square (1 cm²) and multiply it by 30 to obtain the total count per plate. The inoculated area of a Petrifilm Yeast and Mould count plate is approximately 30 cm².

The Petrifilm Yeast and Mould count plate in figure 4 contains a high number of yeast colonies - too numerous to count (TNTC). The small, blue colonies outlined at the edge of the plate differentiate the plate from a TNTC mould count. **(Yeast count = TNTC actual count > 10⁴)**

Sometimes Petrifilm Yeast and Mould count plates with high numbers of yeast colonies may appear to have blue growth only around the edges (figure 5). This is also recorded as a yeast count of TNTC. **(Yeast count = TNTC actual count > 10⁶)**

If Petrifilm Yeast and Mould count plates appear to have no growth, lift the top film and examine the gel that adheres to the top film (figure 6). If numerous yeast are present, you will see white colonies in the gel. This is recorded as a yeast count of TNTC. **(Yeast count = TNTC)**
Moulds

The mould colonies on the 3M™ Petrifilm™ Yeast and Mould Count Plate in figure 7 are variably pigmented colonies, with diffuse edges, and centre foci. They are large, and beginning to crowd, sporulate, and overlap each other on the plate. For ease in counting, divide the plate into sections and look for foci to help distinguish individual colonies. (Mould count = 59) The section shown has 15 moulds.

Note the variable pigmentation, and fuzzy edges of the plate in figure 8, caused by the high numbers of mould colonies and sporulation that has taken place. Estimate the count by counting the foci. There are 4 colonies in the square shown. (Mould count = 120 estimate)

As with all plate count methods, crowded plates may show atypical colony characteristics. Proper dilution is important to ensure an accurate count.

The Petrifilm Yeast and Mould count plates in figures 9 and 10 are 1:10 and 1:100 dilutions respectively, of the same product. The colonies in the figure 9 are small, faint and numerous making the count difficult to estimate. An artifact bubble is present. (Mould count = TNTC)

Dilution of the product to obtain a colony count within the desired counting range (15-150 colonies), makes counting easy. The moulds in figure 10 are large, with diffuse edges and centre foci. (Mould count = 58). The over-crowding on the plate in figure 9 prevented their typical growth.
Phosphatase Reaction

All living cells contain the enzyme phosphatase. In the presence of phosphatase, the indicator in the 3M™ Petrifilm™ Yeast and Mould Count Plates is activated and stains the yeast and mould colonies a blue colour.

Some raw and processed food products that contain living cells (and therefore, phosphatase) may also cause this blue colour reaction to occur. Two types of colour reaction from products are sometimes seen: a uniform blue background colour, or intense, pinpoint blue spots (often seen with spices or granulated products).

A colour reaction caused by natural phosphatase in a product can be distinguished from yeast and mould colonies by one or more of the following techniques:

1. Dilution: When possible, further dilution will eliminate blue background colour, or reduce the number of pinpoint blue spots.
2. Late Supernate: Mix sample and let settle 3-5 minutes to eliminate large product particles that can often cause the pinpoint colour reactions.
3. Incubation Temperature: Incubate plates at the proper temperature 20-25°C. Enzyme (phosphatase) reactions occur faster as temperatures increase.
4. Check & Note: Check Petrifilm Yeast and Mould count plates after 24-48 hours of incubation. Product colour change can occur within 24-48 hours. Make note of any colour seen, to aid in final interpretation.

The 3M™ Petrifilm™ Yeast and Mould Count Plate in figure 11, is an example of a plate with uniform background colour caused by the „natural phosphatase“ present in the sample tested. The „grainy“ appearance is due to particles of product in the dilution plated. To help distinguish from the TNTC yeast or mould count, note the edges of the plate. (Yeast and Mould count = 0)

Figure 12 is an example of intense, pinpoint blue spot reactions seen occurring from the „natural phosphatase“ in some food products. Note their SHAPE - tiny, pinpoints or irregularly shaped, and COLOUR - deep blue, that often look faint, or smeared around the edges of some of the larger particles. (Yeast and Mould count = 0)

Another example of intense blue pinpoint colour reactions is shown in figure 13. The pinpoint dots are very bright, tiny, and irregularly shaped. The yeast colonies are small, blue-green colonies with defined edges. The mould colonies are large, variably pigmented colonies with diffuse edges and centre foci. (Yeast count = 7; Mould count = 7)
Figure 14 is the same product as shown in figure 13, plated after allowing the product particulates to settle 3-5 minutes before plating. There are still a few pinpoint spots (in the squares above) caused by product particles, but most product interference was eliminated.

(Yeast count = 12 Mould count = 4)

**Time & Temperature**

Proper incubation **TIME** and **TEMPERATURE** are important to ensure growth of the types of yeast and mould that can cause spoilage. These yeast and moulds are generally slow growing, and sensitive to high temperatures, regardless of the method used.

To ensure optimum growth, incubate 3M™ Petrifilm™ Yeast and Mould Count Plates at **20°C – 25°C** (room temperature), and check plates for growth at both **3** and **5 days**. Since mould colonies grow between the films, inspecting Petrifilm plates will not dislodge spores and cause additional colonies.

Incubating yeast and mould plates at a higher temperature may not mean a faster result - it may mean an **inaccurate** result as illustrated in the Petrifilm Yeast and Mould count plates in figures 15 and 16. They are duplicate plates of the same product and dilution, but were incubated for different times at different temperatures.

**Yeast count = TNTC**  
Incubated 3 days at 35°C

**Yeast count = TNTC (actual count >107)**  
**Mould count = 120 (estimate)**  
Incubated 5 days at room temperature.
Microscopic Differentiation

Yeasts and moulds are very diverse organisms, and cannot always be distinguished from each other macroscopically. As with any method, positive differentiation can be made with microscopic examination.

To isolate colonies for further identification, lift the top film and pick the colony from the gel.

Transfer the colony to a drop of sterile water on a microscope slide, cover with a coverslip, and view under oil immersion power.

Look for oval shaped, budding YEAST

branching, thread-like filaments (mycelium) – MOULD

or MOULD in various stages of germination.
3M™ Petrifilm™ Yeast & Mould Count Plates

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

1. Refrigerate unopened packages. Use before expiration date on package.
2. To seal opened package, fold end over and tape shut.
3. Keep resealed package at ≤ 21°C (≤ 70°F) and ≤ 50% RH. Do not refrigerate opened packages. Use Petrifilm plates within one month after opening.

Sample Preparation

4. Prepare a 1:10 or greater dilution of food product.* Weigh or pipette food product into Whirl-Pak® bag, stomacher bag, dilution bottle or other appropriate sterile container.
5. Add appropriate quantity of diluent. These include Standard Methods phosphate buffer, 0.1% peptone water, distilled water, phosphate buffered saline, and Butterfield’s buffer. Do not use buffers containing sodium citrate or thiosulfate.
6. Blend or homogenize sample per current procedure.

Inoculation

7. Place Petrifilm plate on level surface. Lift top film.
8. With pipette perpendicular to Petrifilm plate, place 1 mL of sample onto center of bottom film.
9. Release top film; allow it to drop. Do not roll top film down.

* If greater sensitivity is required with dairy or juice products please refer to Petrifilm Dairy & Juice Products sheet.
Inoculation


11. Apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.

12. Lift spreader. Wait one minute for gel to solidify.

Incubation

13. Incubate Petrifilm count plates with the clear side up in stacks of 20 or less at a temperature of 25°C for 3-5 days.

Interpretation

14. Read colonies. A colony counter or any other magnifier light source can be used. Refer to Guide to Interpretation when reading results.

Additional Comments

- Note: Remember to inoculate and spread each Petrifilm plate before going on to the next.
- Steps 9 and 10 are unique to Petrifilm Yeast & Mould count plates.
- Incubate Petrifilm Yeast & Mould count plates in a plastic container or plastic bag to optimize colony development.
The 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent and an indicator system that facilitates yeast and mold enumeration.
Yeast vs. Mold Colonies

To differentiate yeast and mold colonies on the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plates, look for one or more of the following characteristics:

**Yeast**: small colonies, colonies have defined edges, pink-tan to blue-green in color, colonies appear raised (3 dimensional) and colonies have a uniform color.

**Mold**: large colonies, colonies have diffuse edges, blue-green to variable upon prolonged incubation, colonies appear flat and colonies have a dark center with diffused edge.

Growth and Colony Formation

Incubate 3M Petrifilm Rapid Yeast and Mold Count Plates at 25–28°C for 48±2 hours* in a horizontal position with the clear side up in stacks of no more than 40. Certain food types may exhibit clearer growth and colony formation at 28°C.

*If colonies appear faint, allow an additional 12 hours of incubation time for enhanced interpretation. The presence of small air bubbles will not prevent accurate counts.
Enzymatic Reaction

Food samples may occasionally show interference on the 3M Petrifilm Rapid Yeast and Mold Count Plates, for example:

**Figure 3**
Count: 0
A uniform blue background color (often seen from the organisms used in cultured products) should not be counted as TNTC.

**Figure 4**
Count: 5
A uniform blue background color will not prevent an accurate count.

**Figure 5**
Count: 0
A plate without an enzymatic reaction.

**Figure 6**
Count: TNTC
Some foods containing high levels of enzymes may cause a uniform blue background. Colony growth will still be visible if an enzyme reaction occurs.
Reminders for Use: 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

**Inoculation Procedure**

1. Place the 3M Petrifilm Rapid Yeast and Mold Count Plate on a flat, level surface. Lift the top film and with the pipette perpendicular dispense 1mL of sample suspension onto the center of bottom film.

2. Roll the top film down onto the sample.

3. Place the 3M™ Petrifilm™ Flat Spreader (6425) or other flat spreader on the center of the 3M Petrifilm Rapid Yeast and Mold Count Plate.

4. Press firmly on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm Rapid Yeast and Mold Count Plate growth area before the gel is formed. Do not slide the spreader across the film.

5. Remove the spreader and leave the 3M Petrifilm Rapid Yeast and Mold Count Plate undisturbed for at least one minute to permit the gel to form.

6. Incubate 3M Petrifilm Rapid Yeast and Mold Count Plate at 25–28°C for 48±2 hours* in a horizontal position with the clear side up in stacks of no more than 40.

   *If colonies appear faint, allow an additional 12 hours of incubation time for enhanced interpretation.

7. Read yeast and mold results at 48 hours. Certain slower growing yeasts and molds may appear faint at 48 hours. To enhance interpretation of these molds allow for an additional 12 hours of incubation time.

8. 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 (20–25°C/<60% RH) for no longer than 4 weeks.

**Use appropriate sterile diluents:**

- Butterfield’s phosphate buffer (ISO 5541-1), Buffered Peptone Water (ISO), 0.1% peptone water, peptone salt diluent, saline solution (0.85–0.90%), bisulphite-free letheen broth or distilled water.
- Do not use diluents containing citrate, bisulphite or thiosulfate with 3M Petrifilm Rapid Yeast and Mold Count Plates; they can inhibit growth. If citrate buffer is indicated in the standard procedure, substitute with 0.1% peptone water, warmed to 40–45°C.

**3M Food Safety offers a full line of products to accomplish a variety of your microbial testing needs. For more product information, visit us at www.3M.com/foodsafety or call 1-800-328-6553.**

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Petrifilm™ Plates

3M™ 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

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<td>Air or Direct Contact Method</td>
<td>Hydrate plates with 1 mL of appropriate sterile diluent. Allow hydrated plates to remain closed for a minimum of 1 hour before use.</td>
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<tr>
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<td>Hydroplates with 1 mL of appropriate sterile diluent. Allow hydrated plates to remain closed for a minimum of 1 hour before use.</td>
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<tr>
<td>Rapid S. aureus Count</td>
<td>Direct Contact Method Only</td>
<td>Hydroplates with 1 mL of sterile letheen broth only. Place letheen inoculated plates into sealed bag and incubate at 30-37°C (86-99°F) for 24 hours. After incubation, store sealed bag of plates in refrigerator for a minimum of 4 hours to allow gel to solidify. Petrifilm plates hydrated with letheen will have a mottled appearance.</td>
</tr>
</tbody>
</table>

Hydrated Plates Storage Procedures: 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 up to 14 days, all other hydrated Petrifilm plates may be refrigerated up to 7 days.

Air Sampling Method

1. 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.

2. 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.

3. Double-sided tape can also be used with or without clip for positioning of Petrifilm plates for air sampling. Expose Petrifilm plate to air for no longer than 15 minutes. Remove tape and rejoin the top and bottom films.

4. Incubate and enumerate as directed in package inserts. Refer to 3M Petrifilm Plate Interpretation Guide when enumerating results.

Air Sampling Method Results

Petrifilm plate result is count/40 cm² for:
- Aerobic Count
- E. coli/Coliform Count
- Enterobacteriaceae Count

Petrifilm plate result is count/60 cm² for:
- Yeast & Mold Count
- Rapid S. aureus Count

*See relevant Petrifilm plate package insert for details and listing of appropriate diluents. If sanitizers are present, use letheen broth for both the direct contact and swab contact methods.
Direct Contact Method

1. Using a hydrated Petrifilm plate, carefully lift top film. Avoid touching circular growth area. Gel will adhere to top film. Go to step 2a for the surface method or 2b for the finger method.

2a. 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.

2b. Touch finger or portion of hand to hydrated gel area. Rejoin the top and bottom films. Wash hands after finger or hand plating.

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.

3. Incubate and enumerate as directed in package inserts. Refer to 3M Petrifilm Plate Interpretation Guide when enumerating results.

Direct Contact Method Results

Petrifilm plate result is count/\(20 \text{ cm}^2\) for:
- Aerobic Count
- Coliform Count
- Enterobacteriaceae Count
- E.coli/Coliform Count
- Rapid Coliform Count

Petrifilm plate result is count/\(30 \text{ cm}^2\) for:
- Yeast & Mold Count
- Staph Express Count

All Petrifilm plates except Yeast and Mold Count plates and the High-Sensitivity Coliform Count plates can be used for finger or hand plating. The Rapid S. aureus Count plates are not suitable for finger, hand or direct contact method plating.
**Swab Method**

**3M Quick Swab** (wet swabbing method)*

1. Remove the desired quantity of 3M Quick Swabs from the resealable plastic bag. Label the swab.

2. **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.

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

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

5. 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.

6. 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.

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

8. Wring out the contents of the swab tip by pressing and twisting the swab against the wall of the tube.

9. Carefully pour entire contents of the tube onto a 1mL 3M Petrifilm plate. Follow current industry standards for disposal.

10. Incubate and enumerate as directed in package inserts. Refer to 3M Petrifilm Plate Interpretation Guide when enumerating results.

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**Inoculation Procedures**

**1 mL**

**Swab Contact Method Results**

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

**Example**

If area tested was 5 cm² and number of colonies on plate after incubation was 100, your result would be: 100 CFU x 1 mL = 100 CFU/5 cm²

* For 3M Quick Swab dry swabbing method, see Quick Swab package insert.
Inoculation Procedures (continued)

Multi-mL

1. Complete steps 1-6 for the wet swabbing method from previous page.

2. Remove the swab from the tube. Add 1-3 mL’s of sterile diluent to the swab tube. Replace the swab in the tube. Complete steps 7 & 8 of the 1 mL Inoculation Procedure from previous page.

3. 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 new plate until the entire sample is used.

4. Incubate and enumerate as directed in package inserts. Refer to 3M Petrifilm Plate Interpretation Guide when reading results.

Quick Swab Multi-mL Method Results

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

Example
If area tested was 5 cm², number of mLs added was 2 (for total of 3) and number of colonies after incubation was 100, your result would be: 100 CFU x 3 mL = 300 CFU/5 cm²

Alternative Swab Method

Petrifilm plates can be used with other swabbing techniques, however the rinse solution used must be compatible with Petrifilm plates. (See Petrifilm plate package insert for listing of appropriate diluents).

Additional Information

3M Microbiology offers a full line of products to accomplish a variety of your microbial testing needs. For more product information, visit us at www.3M.com/microbiology.

- Canada, call 1-800-563-2921 for technical service.
- Latin America / Africa and Asia Pacific regions, call 1-651-733-7562.

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