Sterile container. bag, dilution bottle, or other appropriate 11/2 inch of the circle's edge may not produce visible gas. Heterofermentative colonies within approximately 1/4 inch of the circle's edge may not be associated with a gas bubble. The colonies in Figure 2 contain both heterofermentative (gas-producing) and non-gas-producing) organisms.

Table 3. Organisms Differentiates Gas Producers from Non-Gas Producers

Table 3. Organisms Differentiates Gas Producers from Non-Gas Producers

Add appropriate quantity of MRS broth diluent. Prepare according to manufacturer's instructions. If necessary the MRS broth diluent may be made using standard diluents including Butterfield's phosphate buffer (0.0425 g/L of KH2PO4 adjusted to pH 7.2), 0.1% peptone phosphate buffer, 0.85-2% saline solution (0.85-% saline solution, peptone salt diluent (ISO method 6887), buffered peptone water (ISO method 6579), distilled water. Blend or homogenize the growth of homo- and heterofermentative lactic acid bacteria.

Interpretation Guide Section.
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.

Both homofermentative (non-gas-producing) colonies (see circle 1) and heterofermentative (gas-producing) colonies (see circle 2) are present.

Artifact bubbles may result from improper inoculation of the Petrifilm plate. They are irregularly shaped and not associated with a colony.

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.

Multiple Testing Procedures

**MRS 2X Concentration Procedure**

1. Prepare MRS broth to a concentration of 2 times (2x) the suggested manufacturer’s quantity.
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 to achieve a 1:20 dilution. plate other microbial tests.

**Sample Preparation**

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.

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

Make a 1:2 dilution of your 1:10 dilution to achieve a 1:20 dilution.

**Inoculation**

1. Prepare Petrifilm plates using plates already prepared (i.e., the GasPak jar plates). The plates are inoculated with the sample until the entire growth area is covered.
2. Place Petrifilm plates into the GasPak jar in a stack of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.

**Interpretation**

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 the 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 the count by 11 for count/gram.

**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 (15g/99 mL or 32g/225 mL). Plate up to 8 mLs for other microbial tests.
3. Add concentrated MRS broth (4x) solution to existing 1:10 dilutions. This will result in half-strength MRS concentration.

**Inoculation**

1. Place 2 mL of the 1:10 sample dilution (0.4 mL, 1:10 dilution + 0.5 mL concentrated MRS = 1:20 dilution) into the GasPak jar in a stack of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.

2. Place plate(s) into the incubator. Incubate Petrifilm plates anaerobically. Place Petrifilm plates in Petrifilm plates may be incubated in the same jar if each stack is separated by a rigid divider.

3. Place Petrifilm plates into the incubator. Incubate Petrifilm plates at 30°-35°C (86°-95°F) for 48 ± 2 h.

**Interpretation**

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 the 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 the count by 11 for count/gram.
**MRS 2X Concentration Procedure**

**Sample Preparation**

1. Prepare MRS broth to a concentration of 2 times (2X) the suggested manufacturer’s quantity.
2. Prepare a 1:10 dilution of the sample with standard diluent. Use the 1:10 dilution to plate other microbial tests.
3. Add concentrated MRS broth solution to existing 1:10 dilution. This will result in half-strength MRS concentration.
4. Place entire Petrifilm plate into the GasPak jar in stacks of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.
5. Incubate Petrifilm plates anaerobically. Place Petrifilm plates into the GasPak jar in place of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.

**Inoculation**

- Plate 1 mL of the 1:20 sample dilution: (0.5 mL 1:10 dilution + 0.5 mL of 2X concentration MRS = 1:20 dilution)
- Incubate Petrifilm plates anaerobically.

**Interpretation**

- Petrifilm plates can be counted on a standard colony counter or other magnified light source. Count all colonies, multiply the count by the dilution factor (20) to determine the number of colonies per mL. 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 (1:19 mL, or 25g/225 mL). Plate up to 8 mLs for other microbial tests.
3. Add concentrated MRS broth solution to existing 1:10 dilution. This will result in half-strength MRS concentration.
   - If the dilution involves adding 1 mL of product to 8 mLs, plate other microbial tests and then add 1 mL of the concentrated MRS solution. Multiply final plate count by 11 for count/gram.
   - If the dilution involves adding 25 mL of product to 225 mL, plate other microbial tests and then add 50 mL of the concentrated 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 = TNTC
Artifact bubbles may result from improper inoculation of the Petrifilm plate. They are irregularly shaped and not associated with a colony.

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

Count = 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.

Multiple Testing Procedures

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 1:12 dilution of your 1:10 dilution to obtain a 1:120 dilution. Plate 1 mL of the 1:120 dilution to obtain a 1:1440 dilution (or 1/1440 of the original sample).

MRS 2X Concentration Procedure

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 (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. Place 1 mL of the 1:10 sample dilution (0.5 mL, 1:10 dilution + 0.5 concentration MRS = 1:20 dilution) into a GasPak jar in the absence of any other growth. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.

5. Incubate Petrifilm plates anaerobically. Place Petrifilm plates in the GasPak jar in the absence of any other growth. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.

6. Petrifilm plates can be counted on a standard colony counter or other magnified light source. Count all colonies, multiply the count by the dilution factor (20) to determine the number of colonies per mL. Refer to the Interpretation Guide Section.
Sample Preparation

1. Prepare at least a 1:10 dilution or a single 1:10 dilution, a multiple microbial test will be run from a single 1:10 dilution, a Multiple Testing Procedure may be more convenient (see other side).

2. Add appropriate quantity of MRS broth diluent to fermenter (or manufacturer's instructions). If multiple microbial tests will be run from a single 1:10 dilution, a Multiple Testing Procedure may be more convenient (see other side).

3. If multiple dilutions can not be made using standard diluents including broth diluents or sterile distilled water, prepare appropriate diluents and aseptically transfer to 20 ml of 0.85% NaCl solution, adjusted to pH 7.3, 1.5% peptone water, peptone water (Difco method), buffered peptone water (Difco method), peptone, or other appropriate diluents. If multiple dilutions are made using standard diluents including broth diluents or sterile distilled water, prepare appropriate diluents and aseptically transfer to 20 ml of 0.85% NaCl solution, adjusted to pH 7.3, 1.5% peptone water, peptone water (Difco method), buffered peptone water (Difco method), peptone, or other appropriate diluents.

4. Prepare at least a 1:10 dilution or a single 1:10 dilution, a Multiple Testing Procedure may be more convenient (see other side). Do not use buffers containing bile, antibiotic, or thiosulfate; they can inhibit growth.

Inoculation

1. Inoculate Petrifilm plates with 1.0 ml or sample according to instructions in package insert.

2. Place Petrifilm plates with the closed side up into the GasPak jar in stacks of no more than 20 plates. Multiple stacks of 20 plates may be incubated in the same jar if each stack is separated by a rigid divider.

Interpretation

1. Petrifilm plates can be counted on a standard Colony Counter or other magnified light source. Count all colonies, multiply the count by the dilution factor to determine the number of colonies per ml of food sample. For all other regions, please refer to page 3.

Additional Comments


- To order 3M Petrifilm Plates in the U.S., call 1-800-328-1671.

- 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.

- For all other regions, please refer to page 3.

Interpretation Guide

- Lactic Acid Bacteria Count v. 2.08

- 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 representative nearest you.

- a. MRS is a common medium for detecting lactic acid bacteria in foods.

- b. A maximum of 30 plates may be incubated.

- c. Normal Lactic Acid Bacteria growth is given in the key to the left. Inoperative organisms are given in the left column. Organisms may be more or less susceptible to the MRS medium. Non-Lactic Acid Bacteria growth is given in the key to the right. For all other regions, please refer to page 3.

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**Petrifilm™ Aerobic Count Plates**

**Lactic Acid Bacteria Method**

For definite CAUTIONS, DISCLAIMER OF WARRANTIES, LIMITED REMEDY, LIMITATION OF LIABILITY, STORAGE AND DISPOSAL, information, and INSTRUCTIONS FOR USE see Product package insert.

3M® Petrifilm™ Aerobic Count Plates can be used to enumerate lactic acid bacteria in certain foods.

- The unique construction of Petrifilm plates makes it possible to distinguish gas-producing heterofermentative organisms from non-gas-producing homofermenters.
- Inoculating a stack of Petrifilm plates, in combination with anaerobic incubation enhances the growth of lactobacilli and other lactic acid bacteria in high acid/high sugar, raw and processed products, such as tomatoes, tomato based products, sauerkraut, sausages, and in processed meat products.

**Sample Preparation**

- Prepare at least 1-10 dilution or from 5 g to 10 mL of the food sample. Weight or aliquot food product into homogenizer, food homogenizer, tube, or other appropriate sterile container.

**Inoculation**

- Add appropriate quantity of MRS broth to Petrifilm plates with manufacturer's instructions. To multiple inoculate, make a single 1/10 dilution. A 1/10 Multiplying Procedure may be used (see other side).

**Incubation**

- Add appropriate quantity of MRS broth to Petrifilm plates and anaerobically incubate. The Petrifilm Aerobic Count plate, in combination with anaerobic incubation, enhances the growth of lactobacilli and other lactic acid bacteria in high acid/high sugar, raw and processed products, such as tomatoes, tomato based products, sauerkraut, sausages, and in processed meat products.

**Interpretation**

- Multiple colonies on the surface of the MRS broth diluent need only be used for the final count. Gas production is indicated by a white, solid, and raised bacterial growth. Gas production is observed within 48 ± 3h.

To order 3M Petrifilm Plates in the U.S., call 1-800-523-7878

Questions? U.S., call 1-800-328-6553

**Organisms**

- **Organisms Gas Producers**
  - Lactobacillus fermentum (gas) +
  - Lactobacillus fermentum (non-gas) +

**Organisms Non-Gas Producers**

- Lactobacillus buchneri
- Lactobacillus delbrueckii subsp. lactis
- Lactobacillus delbrueckii subsp. delbrueckii
- Lactobacillus amylovorus
- Lactobacillus acidiurici
- Lactobacillus casei
- Lactobacillus curvatus
- Lactobacillus plantarum
- Lactobacillus brevis

**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 representative nearest you.

**Interpretation Guide**

**Count = 30**

Multiple colonies form lactic acid producers and non-lactic acid producers. The MRS broth diluent provides a 10-fold enrichment of gas producing bacteria (non-gas-producing) organisms.

**Count < 30**

Up to 3-4 bacteria forming colonies (gas producing and non-lactic acid producers) indicate lower numbers of lactic acid bacteria. The results indicate the presence of non-lactic acid bacteria organisms (see circle 1). Gas production from heterofermentative organisms (see circle 2).

**3M® Microbiology offers a full line of products to accomplish**

- **Sample Preparation**
  - **Inoculation**
  - **Incubation**
  - **Interpretation**

For more information, contact the official 3M Microbiology representative nearest you.