# Heterotrophic Bacteria

DOC316.53.01227

**Pour Plate Method** **Method 8242**

**m-TGE with TTC**

**Scope and Application:** For water and wastewater.

## Test preparation

## Introduction

The Pour Plate Method, also known as the standard plate count, is simple to perform and is commonly used to determine heterotrophic bacteria density. This method does, however, have disadvantages that limit recovery of the maximum number of organisms. Tempered medium at 44–46 °C (111–115 °F) may cause heat shock to stressed bacteria and the nutritionally rich medium may decrease recovery of starved bacteria.

The standard plate count attempts to provide a standardized means of determining the density of aerobic and facultatively anaerobic heterotrophic bacteria in water. Bacteria occur singly or in pairs, chains, clusters or packets, and no single method, growth medium, or set of physical conditions can satisfy the physiological requirements of all bacteria in a water sample. However, the heterotrophic plate count is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of source water.

**Before starting the test:**

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|---|
| See the *Introduction to Bacteria* for more information about preparing sample containers and collecting and preserving samples. |
| To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use. |
| Limit the number of samples to be plated at any one time so that no more than 20 minutes (preferably 10 minutes) elapse between the dilution of the first sample and the pouring of the last plate. |
| To save time, start the incubator before preparing the other materials. Set the incubator for the temperature required in the procedure (usually 35 ± 0.5 °C). |
| Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water. |
| Mark each pour plate, membrane filtration petri dish, or other sample container with the sample number, dilution, date, and any other necessary information. Take care not to contaminate the inside of the sample container in any way. |

## Pour plate procedure for heterotrophic bacteria m-TGE with TTC, method 8242

**1.** Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish. **Do not touch the pad or the inside of the petri dish.**

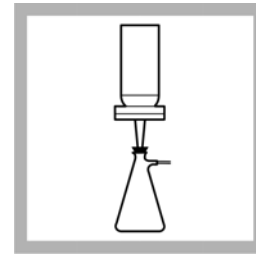
To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

**2.** Invert ampules two or three times to mix broth. Open an ampule of m-TGE with TTC or use an ampule breaker if necessary. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

For broth prepared from dehydrated medium, pipet approximately 2.0 mL of broth onto the pad using a sterile pipet. Drain excess medium from the petri dish and replace the lid.

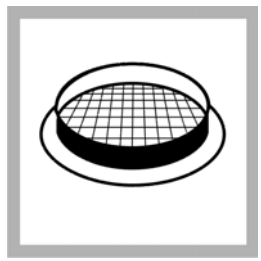
**3.** Set up the Membrane Filter Assembly. Use sterile forceps to place a membrane filter, grid side up, in the assembly.

Alternatively, a sterile, disposable filter unit may be used.



**4.** Invert the sample for 30 seconds, approximately 25 times, to make sure it is well-mixed. Filter the appropriate volume through the sterile 47 mm, 0.45µm, gridded membrane filter. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20–30 mL of sterile buffered dilution water.

**5.** Turn off the vacuum and lift off the funnel top. Remove the membrane filter, using sterile forceps. Still using the forceps, transfer the filter immediately to the previously prepared petri dish.



**6.** With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

**7.** Label the petri dish with the sample number, dilution and date. Invert the petri dish and incubate at 35 ± 0.5 °C for 24 hours.

**8.** Remove the dish from the incubator. Count colonies on membrane filters using a 10–15X microscope.

Bacterial colonies grown on m-TGE with TTC medium appear red to aid visibility.

## Diluting the Sample

The pour plate method requires use of 1 mL, 0.1 mL, and 0.01 mL or 0.001 mL of sample. The difficulty measuring and working with the two smaller volumes, 0.01 and 0.001 mL, require the use of sample dilutions. These dilutions are prepared by pipetting 1 mL of undiluted sample into 99 mL of buffered dilution water. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

## Selecting Sample Volumes/Dilutions

Select the sample volumes or dilutions to be used so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL of undiluted sample, 0.1 mL of undiluted sample and 1 mL of diluted sample (which equals 0.01 mL of undiluted sample). In examining sewage or turbid water, do not measure a 0.1-mL inoculum of the original undiluted sample, but do prepare an appropriate dilution.

## Counting, Computing and Reporting Results

Optimal colony density per filter is 20 to 200. Report all colonies counted as colony forming-units (CFU)/mL. Include in the report the method used, the incubation temperature and time, and the medium.

***For example:*** 98 CFU/L, mL, 35 °C, 24 hours, m-TGE with TTC broth.

**1 to 2, or fewer colonies per square**—Count all of the colonies on the filter, and divide the results by the volume of original sample used.
***For example,*** if there are 122 colonies on the filter, and the volume of original sample used was 10 mL, compute results as follows:

$$\frac{\text{122 colonies}}{\text{10 mL sample}} = 12.2 \text{ CFU/mL}$$

**3 to 10 colonies per square** — Count all colonies in 10 representative squares and divide by 10 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.
***For example,*** if you calculated an average of 8 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{8 colonies/square x 100}}{\text{0.1 mL sample}} = 8000 \text{ CFU/mL}$$

**10 to 20 colonies per square**—Count all colonies in 5 representative squares and divide by 5 to obtain an average number of colonies per square. Multiply this number by 100 and divide by the volume of original sample used.

***For example:*** if there are an average of 17 colonies per square, and the volume of original sample used was 0.1 mL, compute results as follows:

$$\frac{\text{17 colonies/square x 100}}{\text{0.1 mL sample}} = 17,000 \text{ CFU/mL}$$

**More than 20 colonies per square**—If there are more than 20 colonies per square, record the count as > 2000 divided by the volume of original sample used.
***For example,*** if the original volume of sample used were 0.01 mL, results would be > 2000/0.01 or > 200,000 CFU/mL.

***Note:*** *Report averaged counts as estimated CFU/mL. Make estimated counts only when there are discrete, separated colonies without spreaders.*

## Consumables and replacement items

### Required media and reagents

| Description | Unit | Catalog number |
|---|---|---|
| Dilution Water, Buffered, sterile, 99-mL | 25/pkg | 1430598 |
| m-TGE with TTC PourRite™ Ampules, glass, 2-mL | 20/pkg | 2428420 |

### Required apparatus

| Description | Unit | Catalog number |
|---|---|---|
| Absorbent Pads with dispenser, sterile, Gelman | 1000/pkg | 1491800 |
| Ampule Breaker, PourRite™ | each | 2484600 |
| Whirl-Pak Bags with declorinating agent, sterile, 180-mL | 100/pkg | 2075333 |
| Filter Holder, magnetic coupling | each | 1352900 |
| Filtering Flask, 500-mL | each | 54649 |
| Forceps | each | 2141100 |
| Incubator, Culture, low profile, 110 VAC | each | 2619200 |
| Incubator, Culture, low profile, 220 VAC | each | 2619202 |
| Membrane filters, 0.45-µm, gridded, sterile, Gelman | 200/pkg | 1353001 |
| Membrane filters, 0.45-µm, gridded, sterile, Millipore | 150/pkg | 2936100 |
| Microscope, Compound | each | 2942500 |
| Petri Dish, polystyrene, sterile, disposable, without pad | 100/pkg | 1485299 |
| Petri Dish, polystyrene, sterile, disposable, w/pad, Gelman | 100/pkg | 1471799 |
| Petri Dish, polystyrene, sterile, disposable, w/pad, Millipore | 150/pkg | 2936300 |
| Pump, vacuum, 110/115 VAC | each | 2824800 |
| Pump, vacuum, 220/230 VAC, Continental European Plug | each | 2824802 |
| Rubber Stopper, one hole, No. 8 | 6/pkg | 211908 |
| Rubber Tubing, 3.6-m | each | 56019 |
| Pipets, Serological, 10–11 mL, sterile, disposable | 25/pkg | 209798 |

### Optional media, reagents and apparatus

| Description | Unit | Catalog number |
|---|---|---|
| Adapter for rechargeable battery pack, 230 VAC (for 2580300) | each | 2595902 |
| Alcohol Burner | each | 2087742 |
| Aspirator, water | each | 213102 |
| Autoclave, 120 VAC, 50/60 Hz | each | 2898600 |
| Bag, for contaminated items | 200/pkg | 2463300 |

**Optional media, reagents and apparatus (continued)**

| Description | Unit | Catalog number |
|---|---|---|
| Bags, Whirl-Pak®, without dechlorinating agent, 207 mL | 100/pkg | 2233199 |
| Bags, Whirl-Pak®, without dechlorinating agent, 720 mL | 10/pkg | 1437297 |
| Battery eliminator | each | 2580400 |
| Battery pack, rechargeable, for portable incubator 12 VDC | each | 2580300 |
| Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent | 12/pkg | 2599112 |
| Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent | 50/pkg | 2599150 |
| Bottle, sample, sterilized, 100-mL, disposable | 12/pkg | 2495012 |
| Bottle, sample, sterilized, 100-mL, disposable | 50/pkg | 2495050 |
| Counter, hand tally | each | 1469600 |
| Dechlorinating Reagent Powder Pillows | 100/pkg | 1436369 |
| Filter Funnel Manifold, aluminum, 3-place (use with 1352900) | each | 2486100 |
| Filter Unit, sterile, disposable with gridded membrane (use with 2656700) | 12/pkg | 2656600 |
| Filtration Support (for field use), stainless steel | each | 2586200 |
| Funnels, Push-Fit and membrane filters (use with 2586200) | 72/pkg | 2586300 |
| Germicidal Cloths | 50/pkg | 2463200 |
| Incubator, portable, 12 VDC | each | 2569900 |
| Isopropyl alcohol | 500 mL | 1445949 |
| Microscope, Stereo Binocular | each | 2942600 |
| Pump, hand vacuum | each | 1428300 |
| Sterilization Indicator, Sterikon® | 15/pkg | 2811115 |
| Sterilization Indicator, Sterikon® | 100/pkg | 2811199 |
| Wicks, replacement, for alcohol burner 2087742 | — | 2097810 |

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