Pseudomonas

DOC316.53.01214

Pseudomonas Broth Ampules

Method 8026

Membrane Filtration

Scope and application: For potable water, nonpotable water, recreation water and wastewater.

Test preparation

Before starting

Set the temperature of the incubator to 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Wash hands thoroughly with soap and water.
Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.
Make sure that all of the materials that come in contact with samples are sterile.
During filtration, remove the vacuum as soon as the funnel is empty so that the membrane filter does not become dry.
As an alternative to the filter assembly with flask, use a sterile, disposable filter unit.

Items to collect

Description	Quantity
Broth ampule, Pseudomonas	1
Confirmation media	varies
Sterile buffered dilution water	varies
Membrane filter, 0.45 micron	1
Petri dish with absorbent pad, 47-mm	1
Filtration apparatus with aspirator or pump	1
Forceps, sterilized	1
Incubator	1
Microscope, low-power	1
Pipet(s) for dilution or for sample volumes less than 100 mL, if necessary	1

Refer to Consumables and replacement items on page 5 for order information.

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Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. Keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

Sample volumes

Use a sample volume that is applicable to the sample type. For samples with a low level of bacteria such as finished, potable water, use 100 mL of sample. Use less sample for non-potable water or water that contains more bacteria.

When the approximate bacteria level is unknown, analyze three different sample volumes. Use the results from the sample volume that shows approximately 20 to 200 colonies for each membrane filter.

When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

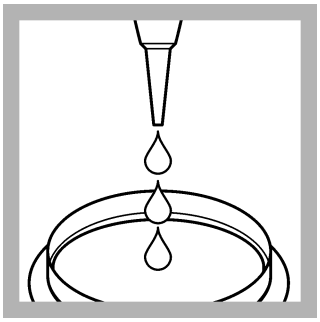
Sample dilution

Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

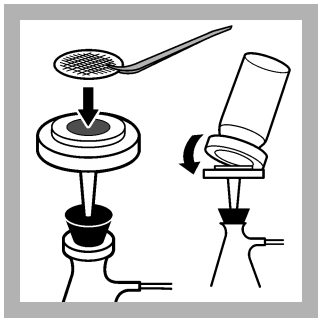
1. Wash hands thoroughly with soap and water.
2. Invert the sample container for 30 seconds (approximately 25 times).
3. Open a bottle of sterile buffered dilution water.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert for 30 seconds (25 times). This is a 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. If necessary, continue to dilute the sample.

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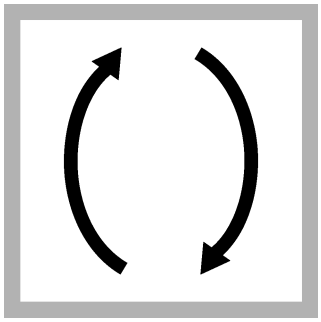
Membrane filtration test procedure



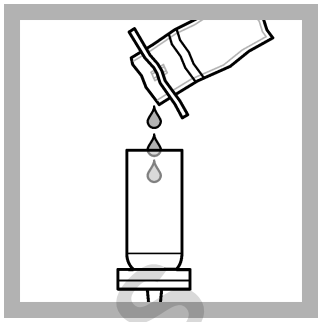
1. Invert one Pseudomonas broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



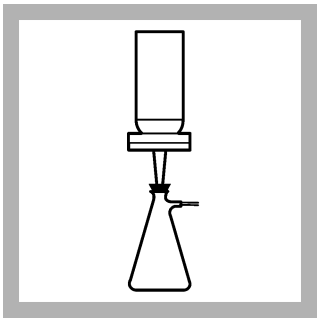
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



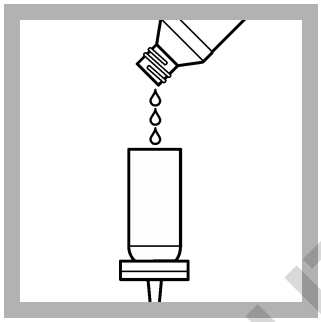
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



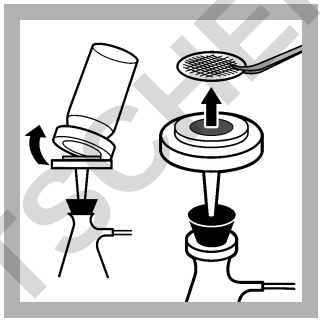
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



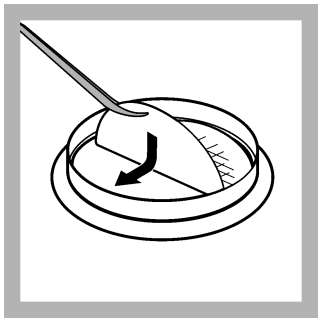
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



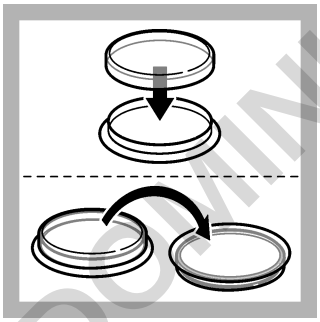
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



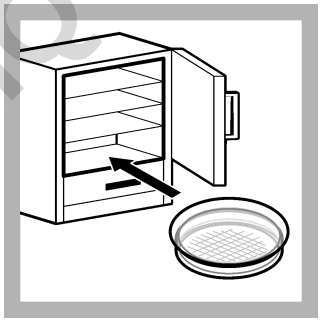
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



8. Put the membrane filter on the absorbent pad. Let the membrane filter bend and fall equally across the absorbent pad to make sure that air bubbles are not caught below the filter.



9. Put the lid on the petri dish and invert the petri dish.



10. Incubate the inverted petri dish at 35 ± 0.5 °C (95 ± 0.9 °F) for 22–24 hours.



11. Remove the petri dish from the incubator. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter. Refer to Interpret and report the results on page 3.

Interpret and report the results

Report the pseudomonas species density as the number of colonies in 100 mL of sample. Use a sample volume that gives 20–80 colonies on the membrane filter.

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If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the pseudomonas species density determination.

1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated colonies.
2. Determine the density as follows:

Membrane filter(s)	Density determination
One membrane filter	Colonies in 100 mL = Colonies counted ÷ mL sample × 100 *Example: 50 colonies were counted. The sample volume was 20 mL. The colony density is 50 ÷ 20 mL × 100 = 250 pseudomonas species in 100 mL of sample.*
Multiple filters, dilutions or duplicates for each sample	Average colonies in 100 mL = Sum of colonies in all samples ÷ sum of mL sample × 100 *Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The colony density is (5 + 9) ÷ (50 + 50) × 100 = 14 pseudomonas species in 100 mL of sample.*

3. If colonies are not isolated or if there are more than 200 colonies of all types:
   a. Report the results as “Confluent growth” when the bacteria grows together across some or all of the membrane filter.
   b. Do the test procedure again with half the sample volume. If the total number of colonies is more than 200 for each membrane or the colonies are not isolated, report the results as “Too numerous to count” (TNTC).
   c. Do the test procedure again with a dilution that gives approximately 50 colonies and not more than 200 colonies of all types.

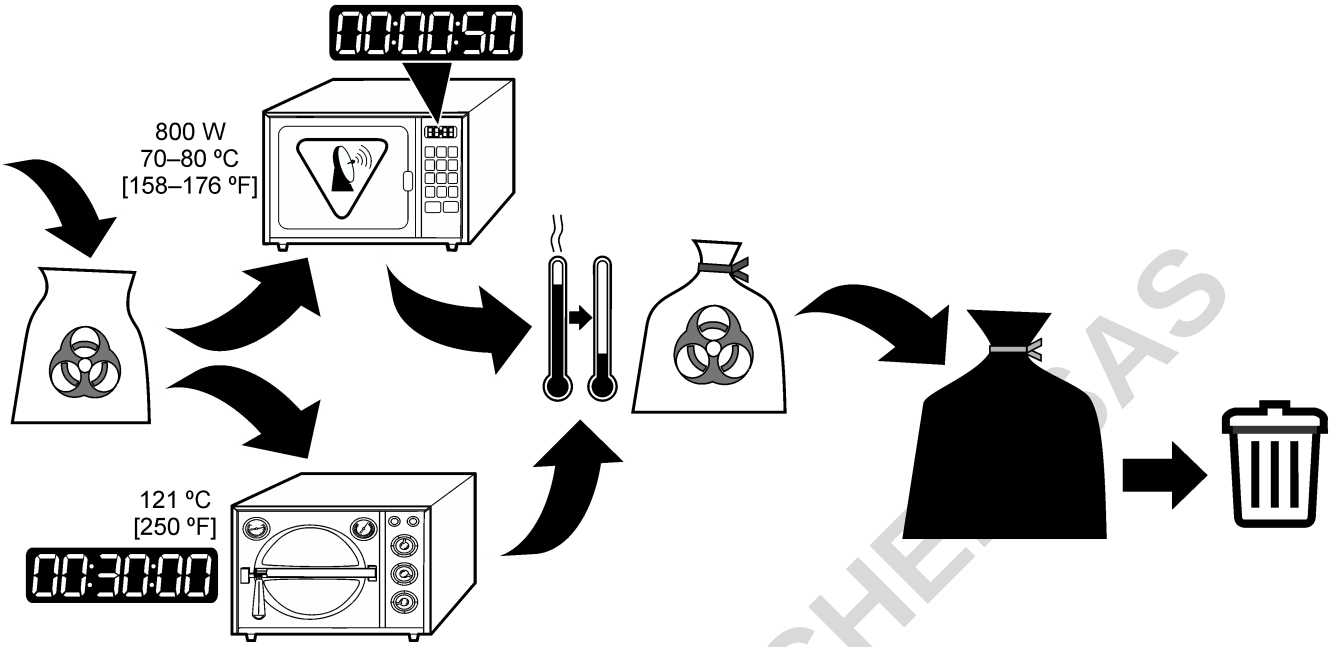
Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1 and the information that follows.

- Microwave—Add 1–2 mL of hypochlorite (bleach) solution to each test container. If a container has a lid, do not close it too tightly. Put the container in the microwave at 70–80 °C (158–176 °F) for 50 seconds. Wait 10 to 15 minutes. Pour the liquid down the drain.
- Autoclave—Put the used test containers in a contaminated items bag or biohazard bag to prevent leaks. Do not seal the bag. Put the bag in the autoclave at 121 °C (250 °F) for 30 minutes at 1.0 bar (15 psi) of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

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Figure 1 Bacteria disposal



Summary of method

Some species of *pseudomonas* bacteria are pathogens that can live in recreational waters such as swimming pools and hot tubs. Pseudomonas Broth is made to isolate species of *pseudomonas*. Pseudomonas Broth has components that help the growth of the pigment pyocyanin, which makes *Pseudomonas aeruginosa* different from other species by the formation of a blue-green, green or yellow-green color. Other species of pseudomonas grow on this medium without the colony color formation.

The membrane filtration procedure is used for samples that are low in turbidity and have low bacteria counts. The sample is poured through a membrane filter. The bacteria in the sample stays on the membrane filter. The membrane filter is moved to a petri dish that contains a nutritional broth or agar. During incubation, the bacteria grow and form colonies on the membrane filter. After incubation, the filter is examined with a microscope for bacteria colonies.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
Pseudomonas broth ampules, plastic	1	50/pkg	2812250
Dilution water, buffered, 99 mL, sterile[1]	1	25/pkg	1430598

Required apparatus

Description	Unit	Item no.
Membrane filter holder, magnetic, 300-mL funnel	each	1352900
Filter pump, aspirator	each	213100
Flask, filtering, glass, 1000 mL	each	54653
Forceps, stainless steel	each	2141100
Membrane filter, 0.45 micron, 47 mm diameter, sterile	200/pkg	1353001

[1] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

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Required apparatus (continued)

Description	Unit	Item no.
Membrane filter, 0.45 micron, 47 mm diameter, sterile EO (ethylene oxide)	150/pkg	2936100
Microscope, compound	each	2947050
Petri dish with absorbent pad, for 47-mm membrane filters, sterile	100/pkg	1471799
Petri dish with absorbent pad, for 47-mm membrane filters, sterile EO (ethylene oxide)	150/pkg	25248000
Stopper, rubber, size 8, for filtration assembly	6/pkg	211908
Pipet, TenSette, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Tubing, rubber, 7.9 mm (5/16-in.) inside diameter	3.66 m (12 ft)	56019

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Portable incubator with 12 VDC power socket	each	2569900
AC power supply for portable incubator, 110–240 VAC	each	2968100
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Portable incubator rack, general purpose/petri dish	each	2580502

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

Optional reagents and apparatus

Description	Unit	Item no.
Disposable filter funnels with membrane filters, sterile	150/pkg	2586300
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet, serological, 2 mL, sterile, glass	35/pkg	2093136
Pipet filler, safety bulb	each	1465100
Support base for disposable filter funnels	each	2586201
Vacuum pump, hand-operated	each	1428300

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