

Heterotrophic Plate count (HPC) Method¹

Method 10289

Peel Plate

Scope and application: For water and wastewater.

¹ Adapted from R2A agar from *Standard Methods for the Examination of Water and Wastewater*, Section 9215.



Test preparation

Before starting

Use the Peel Plate to complete the test in a spread plate or a membrane filtration analysis.
Make sure that all of the materials that come in contact with samples are sterile.
Wash hands thoroughly with soap and water.
Use a dilute bleach solution, bactericidal spray or dilute iodine solution to clean the work area.
Do the test on a level surface in a clean area with no dust and no air drafts at ambient temperature.
Keep the Peel Plates at 0 to 25 °C (32 to 77 °F) in the sealed foil bag with the blue desiccant packets for the maximum shelf life. Note: Refrigeration does not extend the shelf life. The Peel Plates are not shipped refrigerated.
Keep the Peel Plates away from moisture, heat and direct sunlight.
Do not use the Peel Plates if they have changed to yellow or are past the expiration date.
Do not use the Peel Plates if the desiccant packet in the foil bag is not blue (e.g., purple, pink or white).
If the Peel Plates are refrigerated, let the Peel Plates increase to ambient temperature before use. Note: Peel Plates held at room temperature for a minimum of 1 hour open more easily.
Chlorine interferes with the analysis. Add thiosulfate to chlorine disinfected water to remove chlorine interference.
The countable range for the test is 30–300 CFU/mL for unfiltered samples and 20–200 CFU/mL for filtered samples. The recommended target range is 0–80 CFU/mL. Counts that are not within the countable range are estimates and can result in low counts.
Dilute the sample to the countable range. To do a serial dilution, add 10 mL to 90 mL dilution blanks. Multiply the result by the dilution factor.
This method corrects for some variation in pH. The pH of the rehydrated media should be 6.6-7.2 pH.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
Bottle or bag, sterile	1
Volumetric pipette	1
Peel Plate	2
Tweezers	1
Incubator (optional)	1

Refer to [Consumables and replacement parts](#) on page 4 for order information.

Sample collection

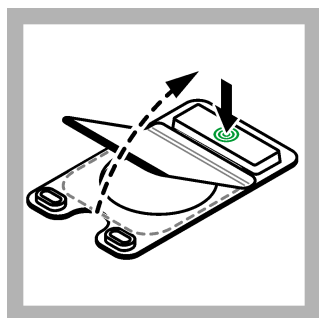
- Use a sterile glass or plastic container such as a Whirl-Pak® bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant. If a Whirl-Pak bag is used, whirl the bag to close and then fold the wire tabs to make a seal. To prevent leaks, do not twist the wire tabs.
- 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 to 3 minutes. Remove any screens or aerators before sample is collected. Do not use faucets or spigots that swivel or leak.
- Keep the Peel Plate tests away from sunlight.
- Collect a minimum of 100 mL of sample and 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 the analysis cannot be started immediately, keep the sample at or below 10 °C (50 °F) for up to 6 hours. Do not let the sample freeze.
- Failure to collect and transport samples correctly will cause inaccurate results.

Test procedure

⚠ CAUTION

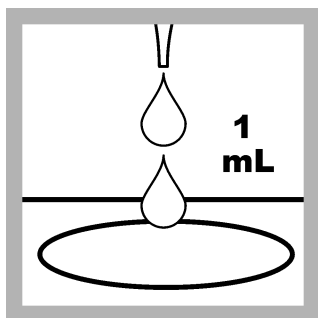


Biological hazard. Obey safety handling protocols and wear all of the personal protective equipment required when in contact with biological hazardous materials.

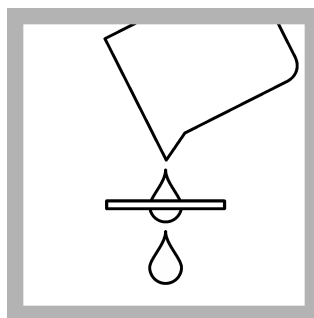


1. Lift the tab and pull the adhesive cover vertically upwards and away to fully have access to the dried culture disc. Keep the cover attached to the back edge of the Peel Plate.

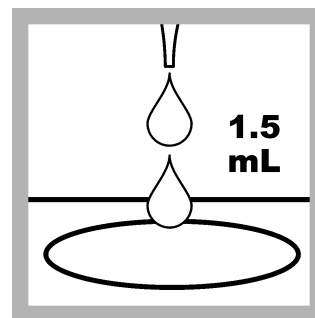
Note: Do not touch the culture disc.



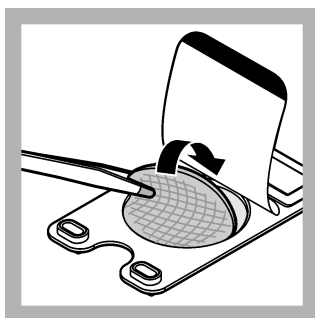
2. Unfiltered samples: Use a pipette to add 1 mL of unfiltered sample to the center of the culture disc. Hold the pipette vertically at an approximate distance of 1–2 cm (0.39–0.79 in) from the culture disc. Add the sample quickly and with constant force. Continue with step 7.



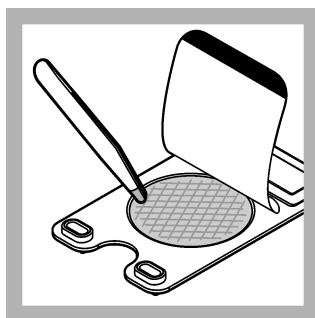
3. Filtered samples: Filter a sample volume with a filtration apparatus with partial vacuum. Use a 47 mm diameter, 0.45 µm pore size membrane filter.



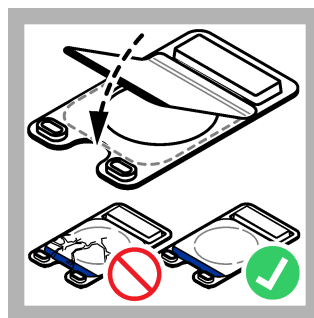
4. Add 1.5 mL of sterile water to the culture disc.



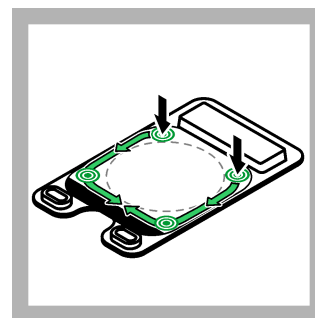
5. Use sterilized tweezers to put the filter with the grid side up on the hydrated culture disc. Place the filter horizontally with a rolling smooth movement to prevent wrinkles on the filter surface and trapping air bubbles.



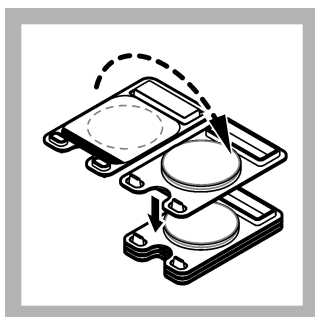
6. To correctly attach the filter, use sterilized tweezers to lightly push the edges of the filter against the Peel Plate disc.



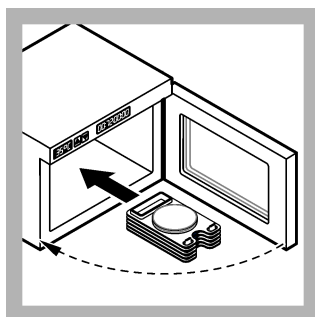
7. Put the adhesive cover on the Peel Plate again. Carefully pull the cover forward to prevent wrinkles.



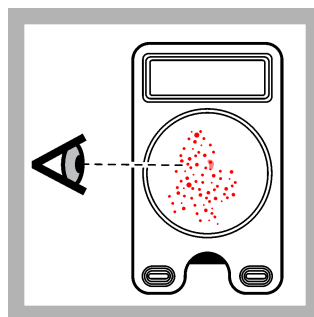
8. Push around the edges of the Peel Plate to make sure of a correct seal of the cover.



9. Turn the Peel Plate with the adhesive cover side down. Stack a maximum of 20 Peel Plate tests.



10. Incubate the Peel Plate tests. The suggested incubation conditions are 5–7 days at 28 °C (82.4 °F).



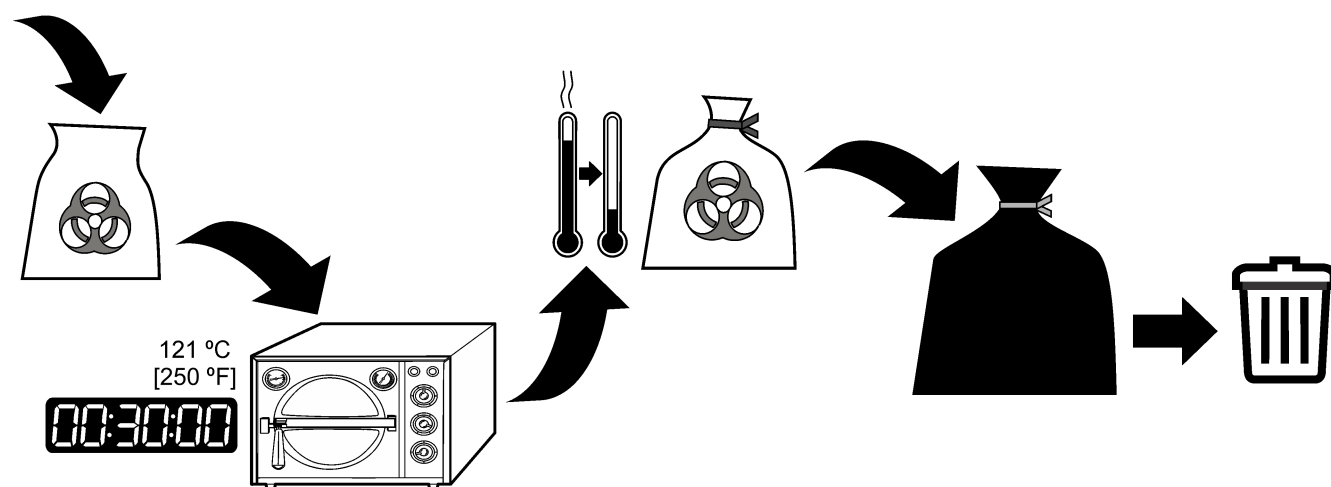
11. Interpret the test results. Count the red spots. Count combined spots as one spot. Each red spot (colony) is 1 CFU/mL for unfiltered samples, or 1 CFU/filtered-water volume (mL) for filtered samples.
Filtered samples: Remove the adhesive cover. Look at the filter under light and magnification at an angle to count the colonies that did not reduce the TTC or form other pigment.

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to [Figure 1](#) to sterilize with an autoclave.

Kill the cultured bacteria with autoclave pressure. 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 15 lb of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

Figure 1 Bacteria disposal



Summary of method

The Peel Plate is a simple procedure that measures the total heterotrophic bacteria numbers in water. Use the heterotrophic plate counts (HPC) test in unfiltered samples and membrane filtered samples. The membrane filtered test is recommended in samples that form very small microcolonies. The Peel Plate medium is based on R2A agar from *Standard Methods for the Examination of Water and Wastewater*, Section 9215. Peel Plate medium also contains the redox-indicator substrate 2,3,5-triphenyltetrazolium chloride (TTC). TTC is reduced by most bacteria to form red colored colonies. The red colored colonies are easier to see against the off-white background.

After incubation, the colonies are counted, and each colony is counted as colony forming unit (CFU).

Consumables and replacement parts

Required media and reagents

Description	Quantity/Test	Unit	Item no.
HPC Peel Plate	1	50/pkg	PP-HPC-50

Required apparatus

Description	Quantity/Test	Unit	Item no.
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Membrane filter, 0.45 micron, 47 mm diameter, sterile (EO ¹)	1	150/pkg	2936100
Tweezers	1	each	1428200

Optional reagents and apparatus

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Sampling bags, Whirl-Pak [®] with dechlorinating agent, 180 mL, sterilized	100/pkg	2075333

¹ Ethylene Oxide sterilization

Optional reagents and apparatus (continued)

Description	Unit	Item no.
Sampling bags, Whirl-Pak [®] without dechlorinating agent, 207 mL	100/pkg	2233199
Filter holder, 47 mm, with receiver	each	2254400



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