Heterotrophic Plate Count Test Methods

Introduction

The Heterotrophic Plate Count tests (HPC) are flexible test methods for the presence and vitality of a diversity of microorganisms. As HPC analyzes measures and indicates pathogen removal and regrowth organisms, they are useful tools for evaluating drinking water samples. HPC provides insight into the effectiveness of water treatment processes, verification of ongoing supply chain quality, and indication of potential system failures. Elevated counts of heterotrophic bacteria can represent a process upset or even a public health hazard. Selection and application of a suitable HPC test can provide valuable understanding of the condition of drinking water throughout the production and distribution system.

The Hach Peel Plate® HPC Test

The Hach® Peel Plate HPC test is a new innovative method developed to streamline the determination of total heterotrophic bacteria numbers in water samples. The Hach Peel Plate test circumvents cumbersome media preparation steps that can lead to erroneous results, wasted materials, and increased labor costs. The Peel Plate HET test is based on Reasoner's 2A agar (R2A agar) from Standard Methods for the Examination of Water and Wastewater, Section 9215. The Hach Peel Plate HET media also contains the redox-indicator substrate 2,3,5-triphenyltetrazolium chloride (TTC). TTC is reduced by most bacteria to produce red colored colonies. It is incorporated into the formulary to facilitate the recognition and counting of colonies against the off-white plate background. The media also contains gelling and wicking agents which absorb and self-wick the sample, eliminating the requirement for manual spreading of the sample.

Figure 1: Step 1 of Hach Peel Plate Method

Performing the Hach Peel Plate Method

Before we begin ... The PeelPlate method can be performed as either a spread plate method with a standard 1 mL sample, or as the media for a membrane filtration test for larger volume analyses. Sample volumes should be adjusted to produce countable results.

Step 1

Place the Peel Plate test onto a level surface. Lift the tab and apply pressure with fingers to the rear rectangular platform as shown in Figure 1.

Step 2

Lift the Peel Plate test tab and pull the adhesive cover vertically upwards and back/away to fully expose the dried culture disc. Leave cover attached to back edge of plate (see Figure 2).

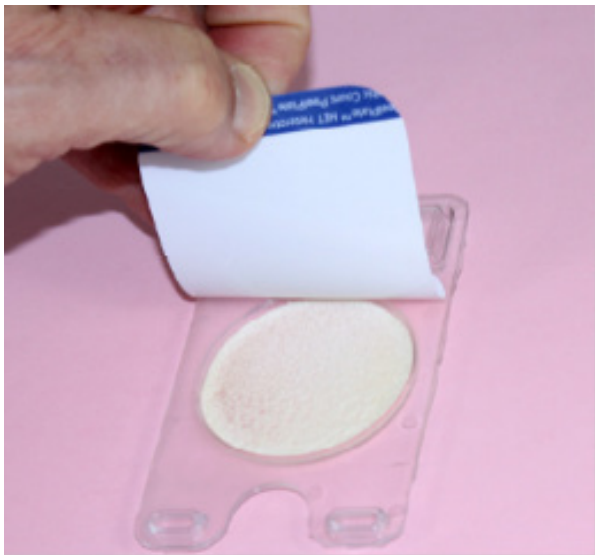

Figure 2: Step 2 of Hach Peel Plate Method

Step 3

Keep the Peel Plate test flat on the surface while holding the cover up. Vertically dispense 1.0 mL of the sample or sample dilution to the center of the exposed Peel Plate disc. Expel pipet contents rapidly with even force and within 2-3 seconds (see Figure 3).

Note: If testing filtered water, re-hydrate the Peel Plate test with 1.5 mL of sterile or microbial free water before applying the membrane filter (see Step 4a).

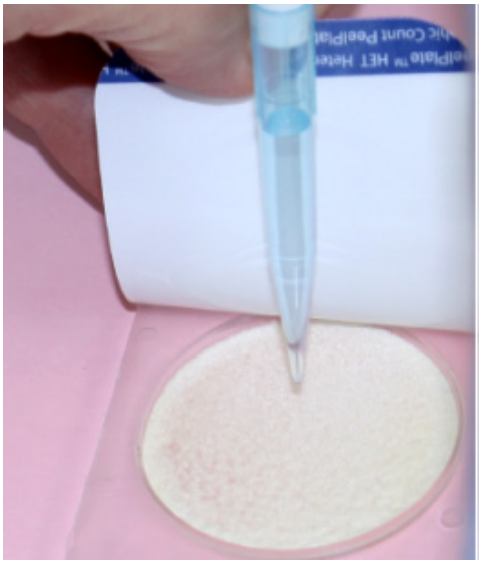

Figure 3: Step 3 of Hach Peel Plate Method

Step 4

The sample will self-wick to the edges of the disc. Reapply the adhesive cover. Press around the edges of the plate to ensure a proper seal. Avoid wrinkling the cover by slightly pulling the cover forward while reapplying to the plate (see Figure 4).

Figure 4: Step 4 of Hach Peel Plate Method

Step 4a: Filter option step

Note that the filtering option may be preferred for samples that traditionally have resulted in micro-colonies as they may be easier to count using this method option.

If testing filtered water, filter the sample through a 0.45 µm, 47 mm mixed cellulose filter membrane using an aspirator under partial vacuum or a syringe. Lift adhesive and pre-wet Peel Plate test with 1.5 mL sterile water. Carefully apply the filter grid side up with tweezers into the plating well. Lay the filter with a rolling motion to avoid trapping air bubbles. Reapply the adhesive cover. Press around edges of the plate to ensure proper seal (see Figure 5).

Figure 5: Step 4a of Hach Peel Plate Method

Step 5

Incubate plates with the adhesive cover side down, grid side up, as shown. Incubate according to standard SOP. Suggested conditions would be 5-7 days at 28°C. Plates can stack by aligning the two pillars and rectangular platform. Plates can be stacked up to 20 high without affecting heat transfer (see Figure 6).

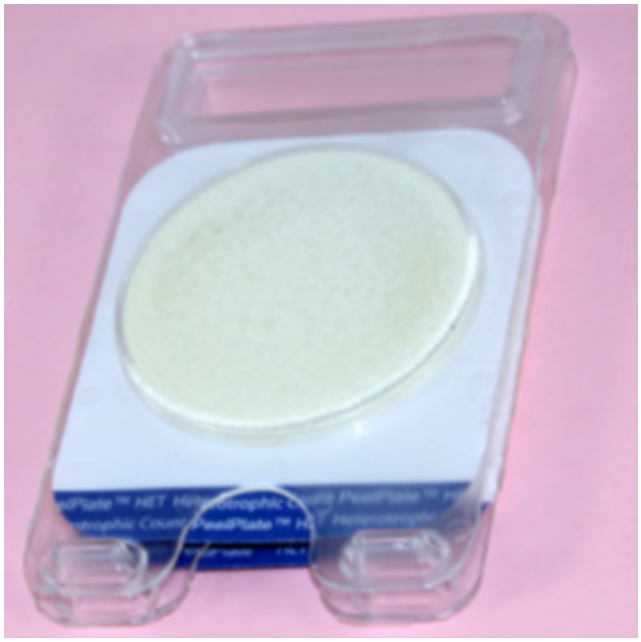

Figure 6: Step 5 of Hach Peel Plate Method

Step 6: Interpreting results

At the end of the incubation period, observe Peel Plate tests for red colonies as viewed though the bottom side of the plate.

- Each spot represents 1 CFU.
- The sum of red spots is reported as the CFU/mL or CFU/filtered-water volume (mL) per sample or sample dilution tested.
- Filtered samples may be viewed from the top-grid side of the filter once the adhesive cover is removed.
- View the filter membranes under illumination and magnification and on an angle to count additional colonies not reducing TTC or producing other pigments.
- In the case of spreading colonies, score a single CFU for each spot and separated color circle.
- Blended colonies may be scored as a single CFU.
- Multiply CFUs by the dilution to calculate a CFU/ mL.

Counts of 30-300 CFU/mL (spread plate) and 20-200 CFU per membrane (filter method) are considered quantitative results, while counts outside of that range are considered estimates. Samples with results outside quantitative range should be diluted or concentrated and retested.

Table 1: Peel Plate test results (after seven days incubation at room temperature in the dark)

Sample Source	Peel Plates 1 mL Direct	Heterotrophic Plate Count Agar	R2A Agar	MTGE TTC Membrane Filtration
Louisiana 1	11, 11, 2	0, 1, 0	6, 10, 7	28, 23
Louisiana 2	TNC, TNC, TNC	TNC, TNC, TNC	TNC, TNC, TNC	TNC, TC
Arizona	0, 1, 5	6, 3, 5	7, 7, 5	1, 0, 0
Louisiana 3	0, 0, 0	1, 0, 0	0, 0, 1	0, 0
Colorado Fridge Filter	TNC, TNC, TNC	TNC, TNC, TNC	TNC, TNC, TNC	290, 275
Louisiana 4	80, 7, 10	0, 0, 8	55, 60, 62	64, 59
Utah	60, SU, 115	TNC, TNC, TNC	TNC, TNC, TNC	TNC, TNC
Southern California	0, 0, 0	0, 0, 0	0, 3, 1	0, 0
Colorado DI	0, 2, 0	16, 19, 26	28, 20, 40	14, 28
Aquarium	40, 41, 47	87, 76, 60	285, 260, 290	28, 35
Open Flowing DI System	1, 0, 0	0, 0, 1	1, 0, 3	3, 0
Loveland DI	TNC, TNC, TNC	145, 210, 160	TNC, 235, TNC	TNC, TNC
Northern California	0, 0, 0	0, 0, 0	0, 0, 2	0, 0
Colorado Toilet Tank	2, 0, 1	0, 0, 0	9, 6, 4	0, 0

[1]Results taken after seven days incubation at room temperature in the dark
[2]TNC is an acronym for too numerous to count (>300)
[3]SU is an acronym for spreader uncountable

Conclusion

With several exceptions, the new Peel Plate method correlates well with the standard R2A method. The pre-made, self-wicking media makes testing for HPC easier and less expensive. The simplicity of the Peel Plate Test makes HPC a convenient analysis for monitoring water quality.

DOC042.53.20238.May16

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