



3M™ Petrifilm™ Aqua Heterotrophic Count Plate
Performance Summary

3M™ Petrifilm™ Aqua Heterotrophic Count (AQHC) Plates are sample ready media plates used in the microbial testing of bottled water. Each plate contains a water-soluble gelling agent, nutrients and indicators in a dry, shelf-stable format.

This technical bulletin summarizes data 3M™ Food Safety collected during performance testing of 3M Petrifilm Aqua AQHC Plates.

Table of Contents

3M™ Petrifilm™ Aqua Heterotrophic Count Plate

Comparative Method Study Results..... p. 2

Inclusivity Study Results p. 5





3M™ Petrifilm™ Aqua Heterotrophic Count (AQHC) Plate Performance Data Comparative Method Study

A comparative method study was conducted at an external reference laboratory¹ to compare results of the 3M Petrifilm Aqua AQHC Plate method to two reference methods for the detection of heterotrophic bacteria in bottled water:

1. ISO 6222: Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium²
2. Standard Methods for the Examination of Water and Wastewater (SMEWW) 9215A6a - Heterotrophic plate count³

Matrices: Fifteen brands of water were tested, two lots per brand, to equal a total of 30 samples.

Table 1: Brands of bottled water tested

Water Type	Brand, Country of Manufacture
Purified	Nestle Pure Life, United States Aquafina, United States Dasani, United States
Regional Spring	Trauth Dairy, United States Ice Mountain, United States Kroger, United States
Natural Spring	Evian, France Jana, Croatia Fiji, Fiji Ty Nant, Wales Voss Flat, Norway
Natural Spring (Carbonated)	Voss Sparkling, Norway Gerolsteiner, Germany Apollinaris, Germany Perrier, France

¹ Q Laboratories, Inc., Cincinnati, OH, USA study comparing 3M Petrifilm Aqua Plate performance vs. reference methods. Study presented at 2011 International Association for Food Protection (IAFP).

² ISO 6222:1999. Water Quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium

³ Standard Methods for the Examination of Water and Wastewater, 20th Ed, 1998, method 9215A6a – NOTE – plate count agar is not recommended in this document for using with filters but it is often used by clients so was considered a modified reference method for this study.



Organisms and Stress Method

Natural spring waters (both carbonated and still) were screened for natural flora. No flora were found in the carbonated natural spring water samples, so those waters were inoculated for the study. Still natural spring waters were found to contain natural flora, and were evaluated both uninoculated as well as being filter-sterilized and inoculated.

For inoculated samples, overnight broth cultures were washed and inoculated in the appropriate bottled water samples at a low/medium inoculum level (targeting 25 cfu/sample) and a medium/high inoculum level (targeting 75 cfu/sample). A non-inoculated control was prepared for each water sample.

The following three organisms were randomly assigned to the 30 different water types: *Enterococcus faecium* ATCC 19434, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 15442. Inoculated water was left in the dark at room temperature for 2 weeks to stress the organisms before testing.

Method Comparison

The following heterotrophic methods were compared:

- a. 3M Petrifilm Aqua AQHC Plate vs. Plate Count Agar (PCA) (100 mL filtered; SMEWW method comparison.) Plates were incubated at $35 \pm 1^{\circ}\text{C}$ for $48 \pm 2\text{h}$.
- b. 3M Petrifilm Aqua AQHC Plate vs. Yeast Extract Agar (YEA) (1 mL direct; ISO method comparison.) Plates were incubated at $36 \pm 2^{\circ}\text{C}$ for $44 \pm 4\text{h}$ and at $22 \pm 2^{\circ}\text{C}$ for $68 \pm 4\text{h}$.

A mixed cellulose ester filter was used for the filtered sample comparison. For each method, samples were plated in duplicate for each level of inoculum. After incubation at the conditions noted above, colonies were counted: all red colonies on 3M Petrifilm Aqua AQHC Plates, all visible colonies on filters on PCA, and all visible colonies on YEA pour plates.

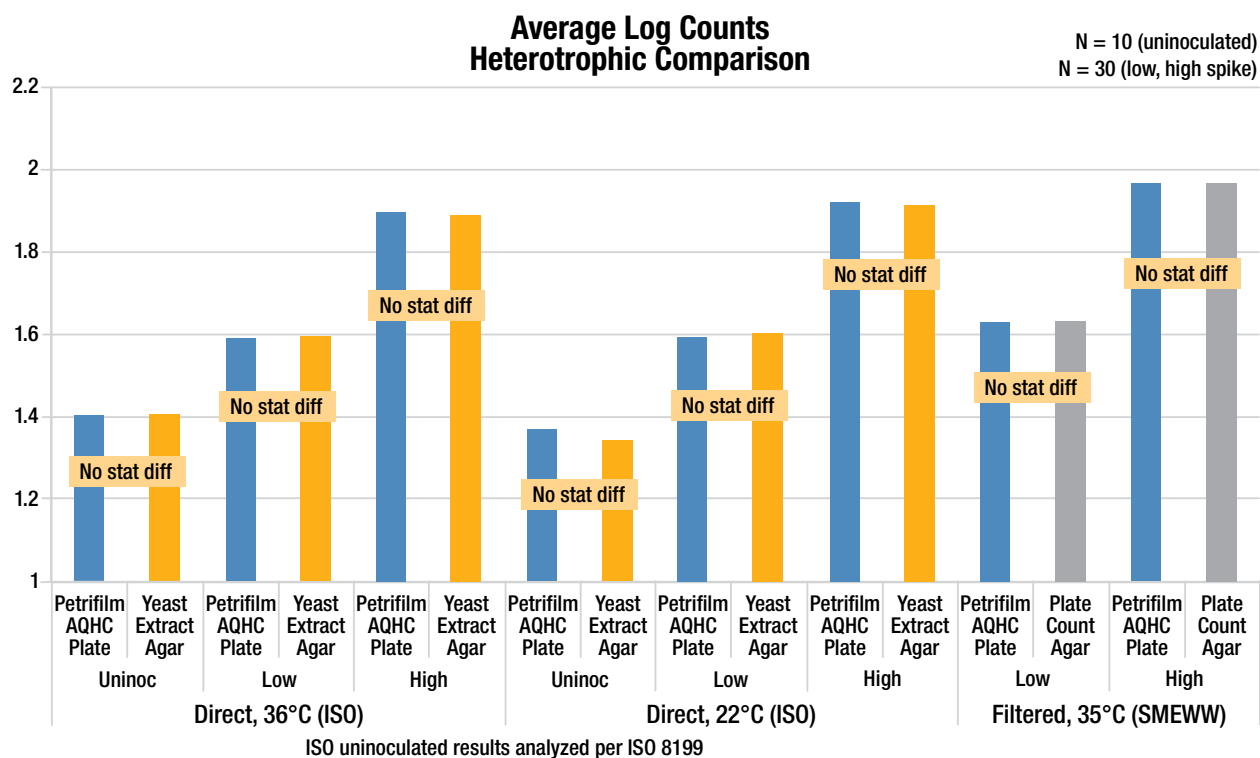
Statistical Analysis

Weighted averages were used for samples in the ISO method comparison per ISO method 8199⁴. For the remaining comparison, counts were converted to $\log_{10}$ counts. A paired t-test by inoculation level was used to compare differences in counts between the 3M Petrifilm Aqua AQHC Plate method and the reference methods. A p-value of <0.05 was taken to indicate a significant difference.

⁴ ISO 8199: 2005(E). Water quality – General guidance on the enumeration of micro-organisms by culture.

Results

Graph 1. Heterotrophic Comparison: Average Log Counts



ISO Method Comparison (direct plating)

- At 36°C, there was no statistical difference between mean log counts on the 3M Petrifilm Aqua AQHC Plate and the YEA method for the uninoculated samples (p-value=0.954), at the low inoculation level (p-value=0.593), or at the high inoculation level (p-value=0.084).
- At 22°C, there was no statistically significant difference between mean log counts on the 3M Petrifilm Aqua AQHC Plate and the YEA method for the uninoculated samples (p-value=0.463), at the low inoculation level (p-value=0.688), or at the high inoculation level (p-value=0.202).

SMEWW Method Comparison (membrane filtration)

- There was no statistical difference between mean log counts per filter on the 3M Petrifilm Aqua AQHC Plate and the PCA at the low inoculation level (p-value=0.833) or the high inoculation level (p-value=0.603).

3M™ Petrifilm™ Aqua Heterotrophic Count (AQHC) Plate Performance Data Inclusivity Study

An inclusivity study was performed using pure cultures of 72 bacterial strains. The strains were obtained from the American Type Culture Collection (ATCC – USA), National Collection of Type Cultures (NCTC – UK), 3M Tecra Culture Collection (TICC – Australia), and isolates from naturally contaminated bottled water and wastewater samples.

Organisms were diluted and inoculated into bottled water targeting 25-150 cfu/sample.

1. One set of inoculated samples was filtered through a mixed cellulose ester filter and plated onto pre-hydrated 3M Petrifilm Aqua AQHC Plates and onto PCA and incubated at $35 \pm 0.5^\circ\text{C}$ for $48 \pm 2\text{h}$.
2. A second preparation of inoculated samples was plated 1 mL direct onto 3M Petrifilm Aqua AQHC Plates and YEA. One set of these plates was incubated at $22 \pm 2^\circ\text{C}$ for $68 \pm 4\text{h}$, and another set was incubated at $36 \pm 2^\circ\text{C}$ for $44 \pm 4\text{h}$.

At the end of the incubation period, all red colonies were counted on 3M Petrifilm Aqua AQHC Plates, and all visible colonies on PCA and YEA were also counted.

Inclusivity Strains

Acidovorax delafieldii 3A1*
Acinetobacter ssp ATCC 49139
Acinetobacter baumannii NCIMB 12457
Actinomyces viscosus ATCC 15987
Aeromonas caviae ATCC 15468
Aeromonas caviae SSW12**
Aeromonas hydrophila ATCC 7965
Aeromonas hydrophila ATCC 35654
Aeromonas hydrophila ATCC 49140
Alcaligenes faecalis ATCC 35655
Alcaligenes faecalis ssp *faecalis* TICC 2709
Bacillus atrophaeus ATCC 51189
Bacillus/Alicyclobacillus/Brevibacillus 8 A1b*
Bacillus pumilus ATCC 14884
Bacillus subtilis subsp. *spizizenii* ATCC 6633
Bacillus subtilis ATCC 11774
Bacillus thuringiensis ATCC 10792
Brevundimonas diminuta TICC 11568
Burkholderia cepacia ATCC 29351
Citrobacter brakii ATCC 29063
Citrobacter freundii TICC 2813
Citrobacter gillenii SSW4**
Comamonas aquatica ATCC 11330
Corynebacterium renale ATCC 19412
Delftia acidovorans 5A4*
Enterobacter aerogenes ATCC 13048

Enterobacter cloacae ATCC 29249
Enterobacter cancerogenus SSW2**
Enterococcus faecium ATCC 51299
Enterococcus faecalis ATCC 14506
Enterococcus faecalis ATCC 700802
Enterococcus faecalis ATCC 7080
Enterococcus saccharolyticus ATCC 43076
Escherichia coli ATCC 25922
Escherichia coli ATCC 13706
Escherichia coli ATCC 51813
Escherichia coli SSW1**
Escherichia coli PWW4**
Flavobacterium ssp ATCC 51823
Granulicatella elegans 3A2*
Hafnia alvei ATCC 51815
Klebsiella pneumoniae ATCC 13882
Klebsiella oxytoca ATCC 51817
Kocuria rhizophila ATCC 9341
Leclercia adecarboxylata ATCC 23216
Moraxella nonliquefaciens ATCC 17953, NCTC 7784
Morganella morganii TICC 4467
Myroides odoratus ATCC 4651
Paenibacillus gordonae ATCC 29948
Proteus mirabilis ATCC 33583
Proteus vulgaris ATCC 33420

Pseudomonas aeruginosa ATCC 27853
Pseudomonas aeruginosa ATCC 15442
Pseudomonas aeruginosa ATCC 7700
Pseudomonas corrugate HPC21*
Pseudomonas fluorescens ATCC 13525
Pseudomonas fluorescens TICC 1356
Pseudomonas putida ATCC 31483
Pseudomonas stutzeri ATCC 17588
Pseudomonas stutzeri 8C1*
Salmonella enterica ser. *Typhimurium* ATCC 51812
Salmonella enterica ser. *Typhimurium* ATCC 13311
Salmonella serotype *Krefeld* TICC 440
Serratia plymuthica ATCC 4475
Shigella sonnei ATCC 29930
Sphingomonas paucimobilis ATCC 29837
Staphylococcus aureus ATCC 6538
Staphylococcus aureus ATCC 25923
Stenotrophomonas maltophilia ATCC 13637
Streptococcus agalactiae ATCC 27956
Streptomyces griseus ATCC 10137
Yersinia enterocolitica ssp *enterocolitica* ATCC 9610

* Strain isolated from bottled water and identified by MicroSEQ® ID identification system.

** Strain isolated from wastewater and identified by API20E or MicroSEQ® ID identification system.



3M™ Petrifilm™ Aqua Heterotrophic Count (AQHC) Plate Performance Data Inclusivity Study (continued)

Inclusivity Results, 22°C, direct samples

3M Petrifilm Aqua AQHC Plates	Yeast Extract Agar
68/72 strains (94.4%) had growth	67/72 strains (93.1%) had growth
Strains that did not grow: <i>Actinomyces viscosus</i> ATCC 15987 <i>Corynebacterium renale</i> ATCC 19412 <i>Kocuria rhizophila</i> ATCC 9341 <i>Moraxella nonliquefaciens</i> ATCC 17953/NCTC 7784	Strains that did not grow: <i>Actinomyces viscosus</i> ATCC 15987 <i>Corynebacterium renale</i> ATCC 19412 <i>Moraxella nonliquefaciens</i> ATCC 17953/NCTC 7784 <i>Staphylococcus aureus</i> ATCC 6538 <i>Staphylococcus aureus</i> ATCC 25923

Inclusivity Results, 36°C, direct samples

3M Petrifilm Aqua AQHC Plates	Yeast Extract Agar
69/72 strains (95.8%) had growth	69/72 strains (95.8%) had growth
Strains that did not grow: <i>Kocuria rhizophila</i> ATCC 9341 <i>Pseudomonas corrugate</i> HPC21 <i>Pseudomonas fluorescens</i> ATCC 13525	Strains that did not grow: <i>Moraxella nonliquefaciens</i> ATCC 17953/NCTC 7784 <i>Myroides odoratus</i> ATCC 4651 <i>Pseudomonas corrugate</i> HPC21

Inclusivity Results, 35°C, filtered samples

3M Petrifilm Aqua AQHC Plates	Plate Count Agar
71/72 strains (95.8%) had growth	68/72 strains (95.8%) had growth
Strains that did not grow: <i>Pseudomonas fluorescens</i> ATCC 13525	Strains that did not grow: <i>Actinomyces viscosus</i> ATCC 15987 <i>Pseudomonas corrugate</i> HPC21 <i>Pseudomonas fluorescens</i> ATCC 13525 <i>Streptococcus agalactiae</i> ATCC 27956

3M and Petrifilm are trademarks of 3M. ©2011.