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

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

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).


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 ± 1°C for 48 ± 2h.

b. 3M Petrifilm Aqua AQHC Plate vs. Yeast Extract Agar (YEA) (1 mL direct; ISO method comparison.) Plates were incubated at 36 ± 2°C for 44 ± 4h and at 22 ± 2°C for 68 ± 4h.

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 log10 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.

---

**Results**

**Graph 1. Heterotrophic Comparison: Average Log Counts**

**Average Log Counts**

**Heterotrophic Comparison**

ISO uninoculated results analyzed per ISO 8199

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).
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 Petrifi lm Aqua AQHC Plates and onto PCA and incubated at 35 ± 0.5°C for 48 ± 2h.

2. A second preparation of inoculated samples was plated 1 mL direct onto 3M Petrifi lm Aqua AQHC Plates and YEA. One set of these plates was incubated at 22 ± 2°C for 68 ± 4h, and another set was incubated at 36 ± 2°C for 44 ± 4h.

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

### Inclusivity Strains

- Acidovorax delafeldii 3A1*
- Acinetobacter baumannii NCIMB 12457
- Actinomyces viscosus ATCC 15987
- Aeromonas caviae ATCC 15468
- Aeromonas caviae SWW12**
- 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 14984
- Bacillus subtilis subsp. spizizenii ATCC 6633
- Bacillus subtilis ATCC 11774
- Bacillus thuringiensis ATCC 10792
- Burkholderia cepacia ATCC 29351
- Citrobacter braak ATCC 29063
- Citrobacter freundii TICC 2813
- Citrobacter gilleni SWW4**
- Comamonas aquatica ATCC 11330
- Corynebacterium renale ATCC 19412
- Delthia acidovorans 5A4*
- Enterobacter cloacae ATCC 29249
- Enterobacter aerogenes ATCC 15442
- Enterobacter aerogenes ATCC 15444
- Enterococcus faecalis ATCC 14506
- Enterococcus faecalis ATCC 700802
- Enterococcus faecalis ATCC 7080
- Escherichia coli ATCC 25922
- Escherichia coli ATCC 13706
- Escherichia coli ATCC 51813
- Escherichia coli SWW1**
- Flavobacterium sp ATCC 51823
- Granulicatella elegans 5A2*
- Hafnia alvei ATCC 51815
- Klebsiella pneumoniae ATCC 13882
- Klebsiella oxytoca ATCC 51817
- Kocuria rhizophila ATCC 9341
- Lecanocereus echinoblastus ATCC 23216
- Morganella morganii ATCC 17953, NCTC 7784
- Morganella morganii TICC 4367
- Myroides odoratus ATCC 10137
- Paenibacillus gordonae ATCC 29948
- Proteus mirabilis ATCC 33583
- Proteus vulgaris ATCC 33420
- Pseudomonas aeruginosa ATCC 27853
- Shigella sonnei ATCC 29930
- Sphingomonas paucimobilis ATCC 29837
- Staphylococcus aureus ATCC 6538
- Staphylococcus aureus ATCC 25923
- Stenotrophomonas maltophilia ATCC 13637
- Streptococcus agalactiae ATCC 27956
- 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.
Inclusivity Results, 22°C, direct samples

<table>
<thead>
<tr>
<th>3M Petrifi lm Aqua AQHC Plates</th>
<th>Yeast Extract Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>68/72 strains (94.4%) had growth</td>
<td>67/72 strains (93.1%) had growth</td>
</tr>
</tbody>
</table>

**Strains that did not grow:**
- Actinomyces viscous ATCC 15987
- Corynebacterium renale ATCC 19412
- Kocuria rhizophila ATCC 9341
- Moraxella nonliquefaciens ATCC 17953/NCTC 7784

**Strains that did not grow:**
- Staphylococcus aureus ATCC 6538
- Staphylococcus aureus ATCC 25923

Inclusivity Results, 36°C, direct samples

<table>
<thead>
<tr>
<th>3M Petrifi lm Aqua AQHC Plates</th>
<th>Yeast Extract Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>69/72 strains (95.8%) had growth</td>
<td>69/72 strains (95.8%) had growth</td>
</tr>
</tbody>
</table>

**Strains that did not grow:**
- Kocuria rhizophila ATCC 9341
- Pseudomonas corrugate HPC21
- Pseudomonas fluorescens ATCC 13525

**Strains that did not grow:**
- Moraxella nonliquefaciens ATCC 17953/NCTC 7784
- Myroides odoratus ATCC 4651
- Pseudomonas corrugate HPC21

Inclusivity Results, 35°C, filtered samples

<table>
<thead>
<tr>
<th>3M Petrifi lm Aqua AQHC Plates</th>
<th>Plate Count Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>71/72 strains (95.8%) had growth</td>
<td>68/72 strains (95.8%) had growth</td>
</tr>
</tbody>
</table>

**Strains that did not grow:**
- Pseudomonas fluorescens ATCC 13525

**Strains that did not grow:**
- Actinomyces viscous ATCC 15987
- Pseudomonas corrugate HPC21
- Pseudomonas fluorescens ATCC 13525
- Streptococcus agalactiae ATCC 27956

3M and Petrifi lm are trademarks of 3M. ©2011.