3M | Petrifilm™

Technical Bulletin

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Technology Platform:
3M™ Petrifilm™
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St. Paul, MN

3M™ Petrifilm™ Yeast and Mold Count Plate

Performance Summary

3M™ Petrifilm™ Yeast and Mold Count Plates are sample-ready media plates used for the enumeration of yeast and mold in the food and beverage industries. 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 Yeast and Mold Count Plates for microbial testing of bottled water.

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Comparative Method Study

A method comparison study was conducted at an external reference laboratory to compare results of the 3M Petrifilm Yeast and Mold Count Plate method to a reference method, Chlortetracycline Rose Bengal[1] (CRB) agar and to another agar, acidified Potato Dextrose Agar (aPDA), for the recovery of yeast and mold in bottled water.

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

Organisms

Overnight broth cultures were washed and inoculated into 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 also prepared for each water sample.

The following three organisms were randomly assigned to the 30 different water types: *Penicillium sp.* ATCC #18307, *Paecilomyces sp.* ATCC #1114, and *Candida albicans* ATCC #10231. Inoculated water was left in the dark at room temperature overnight to equilibrate before filtering.

[1] Standard Methods for Examination of Water and Wastewater (SMEWW) method 9610D 2a - Membrane filter technique for detection of fungi, 20th Edition, 1998.

3M Petrifilm Yeast and Mold Count Plate Performance Data

Comparative Method Study (continued)

Method Comparison

The following yeast and mold methods were compared:

- 3M Petrifilm Yeast and Mold Count Plates vs. Chlortetracycline Rose Bengal [CRB] agar (100 mL filtered; SMEWW). Plates were incubated at 20 ± 1 °C and counted at 3 & 5 days.
- 3M Petrifilm Yeast and Mold Count Plates vs. acidified Potato Dextrose [aPDA] agar (100 mL filtered; customer method). Plates were incubated at 25 ± 1 °C and counted at 3 & 5 days.

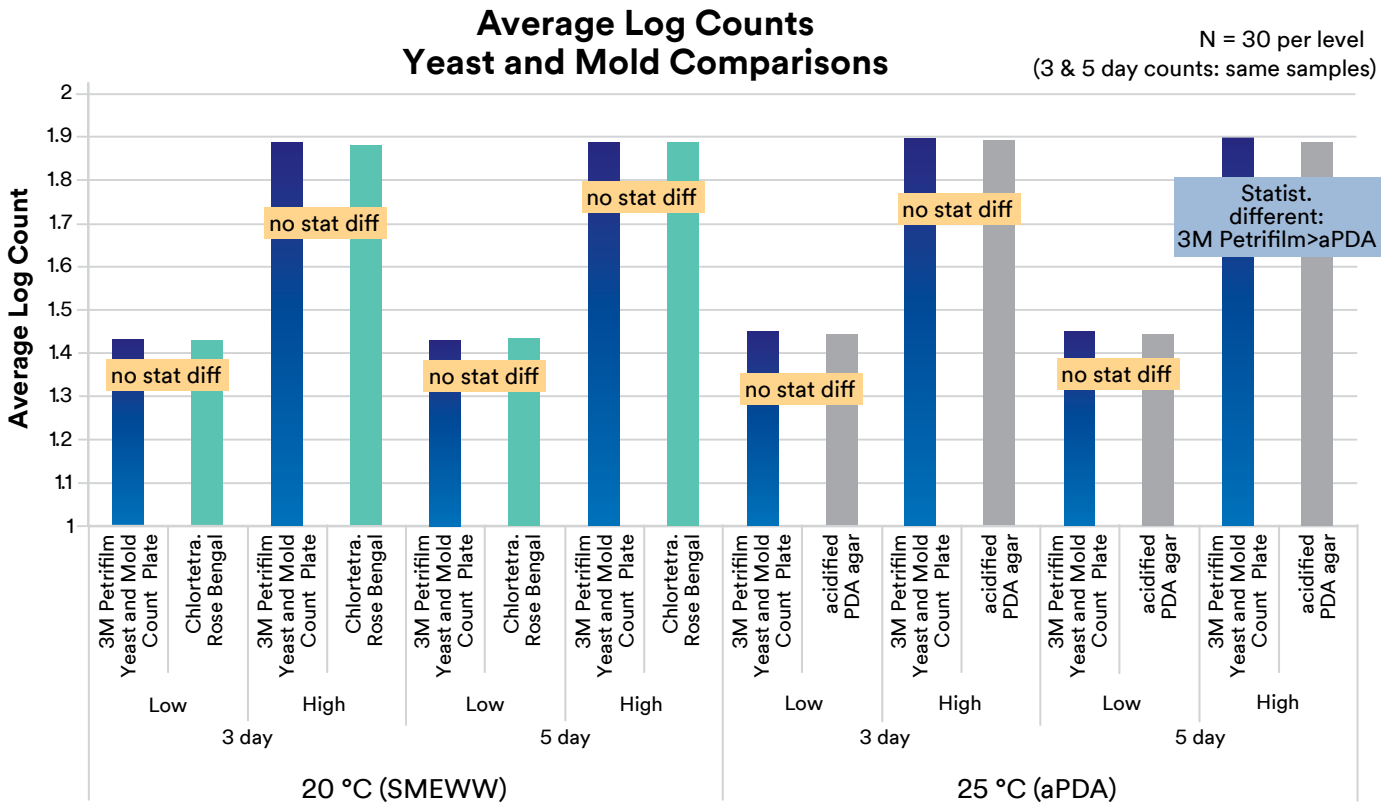
A mixed cellulose ester filter was used for the method comparison. Samples were plated in duplicate for each level of inoculum. After incubation at the conditions noted above, all colonies on all plates were counted.

Statistical Analysis

Counts were converted to $\log_{10}$ counts. A paired t-test per inoculation level was used to compare differences in counts between the 3M Petrifilm Yeast and Mold Count Plate method and the comparative methods. A p-value of <0.05 was taken to indicate a significant difference.

Results

Graph 1: Yeast and Mold Comparisons: Average Log Counts



SMEWW method comparison

- The mean log yeast and mold counts were not significantly different between the 3M Petrifilm Yeast and Mold Count Plate method and the CRB agar method at the low inoculation level at day 3 (p-value=0.745) or the high inoculation level at day 3 (p-value=0.063).

- The mean log yeast and mold counts were not significantly different between the 3M Petrifilm Yeast and Mold Count Plate method and the CRB agar method at the low inoculation level at day 5 (p-value=0.121) or the high inoculation level at day 5 (p-value=0.052).

Customer method comparison

- The mean log yeast and mold counts were not significantly different between the 3M Petrifilm Yeast and Mold Count Plate method and the aPDA method at the low inoculation level at day 3 (p-value=0.844) or the high inoculation level at day 3 (p-value=0.723).

- The mean log yeast and mold counts were not significantly different between the 3M Petrifilm Yeast and Mold Count Plate method and the aPDA method at the low inoculation level at day 5 (p-value=0.506).

- At the high inoculation level at day 5, the mean log yeast and mold counts per filter were significantly different between the 3M Petrifilm Yeast and Mold Count Plate method and the aPDA method (p=0.036), with the log counts per filter for the 3M Petrifilm Yeast and Mold Count Plate method, on average, greater in value than those from the aPDA method.

3M Petrifilm Yeast and Mold Count Plate Performance Data

Inclusivity/Exclusivity Study

Inclusivity and exclusivity studies were performed in an external and internal study that combined consists of 19 fungal and 19 non-fungal pure culture strains. The strains were obtained from the American Type Culture Collection (USA) or from the 3M culture collection. Fungal organisms were diluted and inoculated into bottled water targeting 25-50 cfu/filter; non-fungal strains were diluted and inoculated targeting approximately 1×10^5 cfu/filter.

Inoculated samples were filtered through a mixed cellulose ester filter and plated onto 3M Petrifilm Yeast and Mold Count Plates, Chlortetracycline Rose-Bengal agar (CRB) or acidified potato dextrose agar (aPDA).

1. One set of 3M Petrifilm Yeast and Mold Count Plates and the CRB plates were incubated at 20 ± 1 °C for 5 days, reading at both 3 and 5 days.

2. A second set of 3M Petrifilm Yeast and Mold Count Plates and the aPDA plates were incubated at 25 ± 1 °C for 5 days, reading at both 3 and 5 days.

3. Non-fungal strains were also filtered and plated onto plate count agar and incubated at 35 ± 1 °C for 48 ± 2h as a positive control.

Inclusivity Strains

Alternaria alternaria	ATCC 6663
Aspergillus brasiliensis	ATCC 16404
Aspergillus niger	M6 (3M)
Candida albicans	ATCC 10231
Candida albicans	ATCC 66027
Candida glabrata	ATCC 26512
Candida guilliermondii	ATCC 6260
Chaetomium globosum	ATCC 6205
Cladosporium cladosporioides	ATCC16022
Hansenula (Pichia) anomala	ATCC 2349
Paecilomyces variotii	ATCC 1114
Penicillium corylophilum	ATCC 18307
Pityrosporum ovale	ATCC 12078
Rhizopus stolonifer (+)	ATCC 6227b
Rhodotorula mucilaginosa	ATCC 9449
Saccharomyces cerevisiae	ATCC 18824
Saccaromyces cerevisiae	ATCC 2601
Scopulariopsis acremonium	ATCC 58636
Trichosporon mucoides	ATCC 204094

Exclusivity Strains

Acinetobacter baumanii	ATCC 19606
Aeromonas hydrophila	ATCC 7965
Bacillus atrophaeus	ATCC 51189
Bacillus cereus	ATCC 11774
Bacillus pumilis	ATCC 14884
Enterobacter aerogenes	ATCC 13048
Enterococcus faecalis	ATCC 29212
Escherichia coli	ATCC 25922
Enterococcus faecalis	ATCC 29212
Escherichia coli	ATCC 25922
Lactobacillus delbrueckii subsp. lactis	ATCC 4797
Micrococcus luteus	ATCC 10240
Pseudomonas aeruginosa	ATCC 15442
Pseudomonas fluorescens	ATCC 13525
Salmonella enteridis typhimurium	ATCC 13311
Salmonella enterica	ATCC 51812
Sphingomonas paucimobilus	ATCC 29837
Staphylococcus aureus	ATCC 25923
Yersinia enterocolitica	ATCC 9610

Results Comparison to SMEWW Method, 20 °C

Inclusivity

	3M Petrifilm Yeast and Mold Count Plates	CRB Agar
3 days	15/19 strains (79%) had growth	15/19 strains (79%) had growth
	Strains with no visible growth: *Alternaria alternaria* ATCC 6663 *Chaetomium globosum* ATCC 6205 *Cladosporium cladosporiodes* ATCC 16022 *Scopulariopsis acremonium* ATCC 58636	**Strains with no visible growth:** *Alternaria alternaria* ATCC 6663 *Chaetomium globosum* ATCC 6205 *Cladosporium cladosporiodes* ATCC 16022 *Scopulariopsis acremonium* ATCC 58636
5 days	19/19 strains (100%) had growth	19/19 strains (100%) had growth

Exclusivity

	3M Petrifilm Yeast and Mold Count Plates	CRB Agar
5 days	4/19 strains (21%) had growth	1/19 strains (5%) had growth
	Strains with visible growth: *Acinetobacter baumanii* ATCC 19606 (1 colony) *Aeromonas hydrophila* ATCC 7965 *Enterobacter aerogenes* ATCC 13048 (1 colony) *Enterococcus faecalis* ATCC 29212 (1 colony)	**Strains with visible growth:** *Aeromonas hydrophila* ATCC 7965

Results Comparison to Customer Method, 25 °C

Inclusivity

	3M Petrifilm Yeast and Mold Count Plates	aPDA Agar
3 days	5/19 strains (79%) had growth	15/19 strains (79%) had growth
	Strains with no visible growth: *Alternaria alternaria* ATCC 6663 *Chaetomium globosum* ATCC 6205 *Cladosporium cladosporiodes* ATCC 16022 *Scopulariopsis acremonium* ATCC 58636	**Strains with no visible growth:** *Alternaria alternaria* ATCC 6663 *Chaetomium globosum* ATCC 6205 *Cladosporium cladosporiodes* ATCC 16022 *Scopulariopsis acremonium* ATCC 58636
5 days	20/20 strains (100%) had growth	18/19 strains (95%) had growth
		Strains with no visible growth: *Scopulariopsis acremonium* ATCC 58636

Exclusivity

	3M Petrifilm Yeast and Mold Count Plates	aPDA Agar
5 days	4/19 strains (21%) had growth	1/19 strains (5%) had growth
	Strains with visible growth: *Aeromonas hydrophila* ATCC 7965 *Bacillus atrophaeus* ATCC 51189 (1 colony) *Bacillus cereus* ATCC 11774 (1 colony) *Salmonella enteridiidis* ATCC 13311 (1 colony)	**Strains with visible growth:** *Aeromonas hydrophila* ATCC 7965

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