

# P3-15: Rapid Quantitative Enumeration of Yeasts and Molds

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## ABSTRACT

Yeast and Molds are ubiquitous food spoilage agents that can grow over a wide range of temperatures, pH values and more importantly at reduced water activities ( $a_w$ ), thus having the potential for substantial economic losses to the food industry. These organisms have become important indicators for monitoring food quality, however, the 5–7 day incubation required by traditional enumeration methods poses a burden on food producers. A novel dehydrated film medium (3M™ Petrifilm™ Rapid Yeast and Mold Count Plate) was developed to address the need for rapid (48–60 hours) detection of yeasts and molds. The detection technology was optimized to overcome the inherent limitations of yeast and mold appearance and interpretation on dehydrated media.

## STUDIES AND RESULTS

### INCLUSIVITY ORGANISMS

Performance of three lots of 3M Petrifilm Rapid Yeast and Mold Count Plates at 25°C and 28°C was compared to reference media commonly used for yeast and mold quantification (Dichloran Rose Bengal Agar — DRBC and acidified Potato Dextrose Agar-aPDA). The study involved a total of 38 fungal strains which included 21 yeast and 22 mold isolates. Counts obtained at 48 hours and 60 hours on the 3M Petrifilm Plate were statistically compared to results obtained from the reference media after five days at 25±1°C using regression and one-way ANOVA analysis.

### EXCLUSIVITY ORGANISMS

*Enterococcus faecalis*, *Bacillus spizizenii*, *Aeromonas hydrophila* and *Escherichia coli* were tested as representative Gram positive and Gram negative bacteria to demonstrate the selectivity of three lots of 3M Petrifilm Rapid Yeast and Mold Count Plates against bacteria.

## FOOD STUDIES

Studies were conducted to assess the performance of the method in the presence of potential interferences from sample matrix and other organisms. 42 foods were purchased from retail sources. The foods were tested for indigenous fungal populations using the 3M Petrifilm Rapid Yeast and Mold Count Plates and the reference methods (FDA-BAM; ISO 21527: Parts 1&2). Five yeast species and three mold species were used to artificially contaminate food samples which had very low or undetectable indigenous fungal populations. Pre-prepared media (DRBC, DG-18) and reagents (0.1% Peptone Water) used for the dilution plate method were purchased from Hardy Diagnostics. Sample suspensions and dilution were made in 0.1% Peptone Water. 1mL portions of each dilution were inoculated on the 3M Petrifilm Rapid Yeast and Mold Count Plates and 0.1mL portions were inoculated on DRBC or DG18 agar in triplicate. DRBC agar was used for foods with water activity ( $a_w$ ) equal to or higher than 0.95, whereas DG18 agar was utilized when dried food ( $a_w < 0.95$ ) were tested. A set of 3M Petrifilm Rapid Yeast and Mold Count Plates were incubated upright at 25°C and a second set at 28°C. Both sets of 3M Petrifilm Rapid Yeast and Mold Count Plates were counted after an incubation period of 48 hours and 60 hours.

## ENVIRONMENTAL STUDIES

Environmental samples were evaluated using air, swab and direct surface contact surface methodologies, with each of the three lots of the 3M Petrifilm Rapid Yeast and Mold Count Plates. The plates used for air and direct-contact surface samples were hydrated with 1mL 0.1% Peptone Water.

Environmental swab samples were obtained using 3M™ Quick Swab with Lethen Broth. Several swabs were taken within an area, pooled and plated on the 3M Petrifilm Plates and PDA w/chloramphenicol as the reference media. The plates used for Air Sampling were hydrated with 1mL 0.1% Peptone Water and were allowed to sit at ambient temperature for one hour before sampling.

## RESULTS

Table 1: List of Foods Tested with the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plates

Pies/Bakery	Juices	Seafood	Meat/Poultry	Nuts	Dairy	Fruits/Vegetables	Miscellaneous
– Frozen Apple Pie – Key Lime Pie – Chocolate Dipped Cream Puffs – Chocolate Fudge Topping – Frozen Rolls – Blue Raspberry Powder – All Purpose Flour – Unbleached All Purpose Flour	– 100% Apple – Cranberry Cocktail – Grape – Orange – Orange (Calcium/Vitamin D) – Strawberry/Kiwi Capri Sun	– Smoked Salmon – Swordfish – Tuna – Cooked Salad Shrimp	– Turkey – Ground Beef	– Cashews – Pecans – Walnuts	– Shredded Mild Cheddar Cheese (3 Brands) – Sour Cream (3 Brands) – Greek Yogurt – Light Vanilla Yogurt – Vanilla Yogurt – Activia (Probiotic) – Instant Non Fat Dry Milk	– Organic Blueberries – Whole Strawberries – Sweet Peas – Mixed Vegetables	– Egg White

Figure 2: Comparison of 48 Inclusivity Strains of Yeasts and Molds on Three Lots of 3M™ Petrifilm™ Rapid Yeast and Mold Plates vs. Reference Method at 25°C and 28°C

Figure 2A: Comparison to DRBC at 25°C

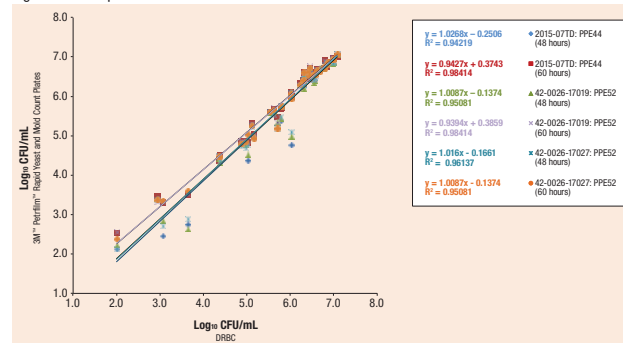


Figure 2B: Comparison to Acidified Potato Dextrose Agar at 25°C

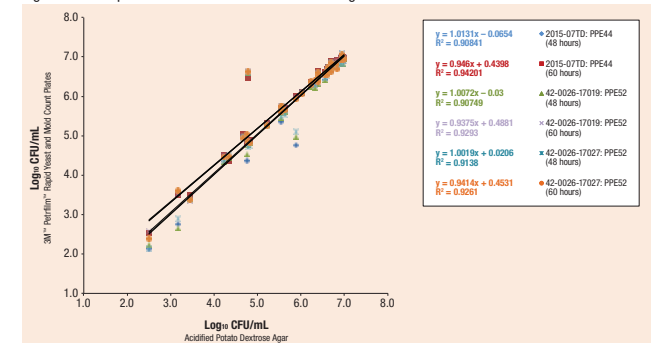


Figure 2C: Comparison to DRBC at 28°C

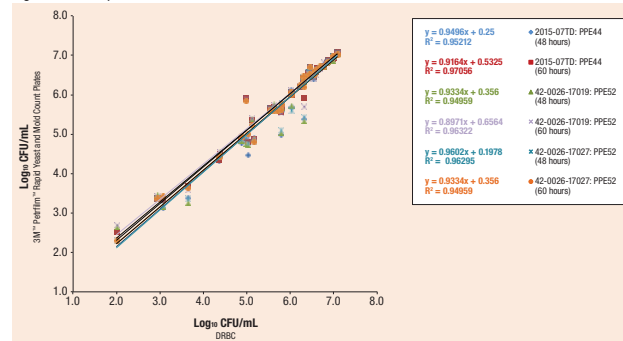
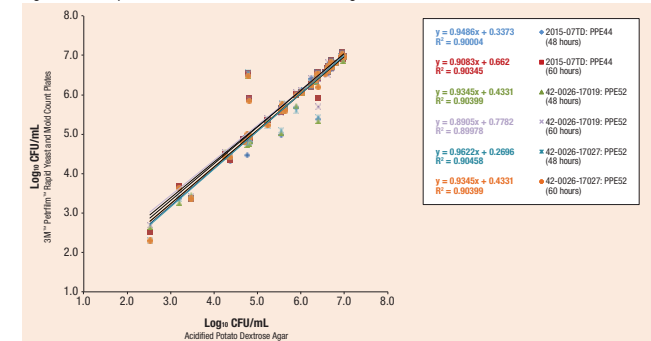


Figure 2D: Comparison to Acidified Potato Dextrose Agar at 28°C



Results continued on back page

## CONCLUSIONS

- Quantitative results obtained on the 3M Petrifilm Rapid Yeast and Mold Count Plates at 48 hours were statistically comparable to those obtained on the Dichloran Rose Bengal Agar and acidified Potato Dextrose Agar in five days.
- The detection technology performed comparably at both 25°C and 28°C incubation temperatures.
- The 3M Petrifilm Rapid Yeast and Mold Count Plates provide the end user with actionable results within 48–60 hours which is a greater than 50% savings in time to result, compared to the reference method.

## ACKNOWLEDGEMENTS

We would like to thank Sara Halaszi, Ashley Smith and Ayele Erabo for the contributions to the laboratory experimentation.

RESULTS (cont.)

Figure 1: Appearance of Yeast and Molds on the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plates

Figure 1A: Smoked Salmon with *Candida tropicalis* (25°C, 48 hours)

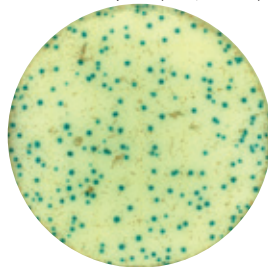


Figure 1B: Non Fat Dry Milk with *Geotrichum candidum* (25°C, 48 hours)

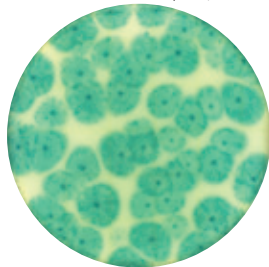


Figure 1C: Cranberry Juice Cocktail with *Saccharomyces cerevisiae* (25°C, 48 hours)

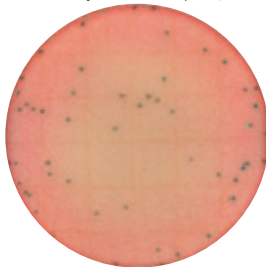


Figure 1D: Cold Brew Green Tea — Naturally Contaminated (28°C, 48 hours)

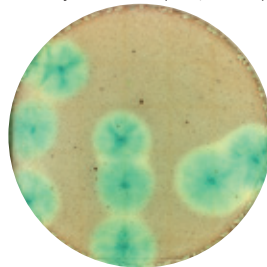


Figure 1E: Cashews with *Aspergillus japonicus* (28°C, 48 hours)

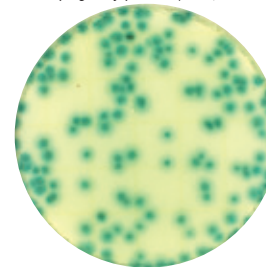


Figure 1F: Walnuts — Naturally Contaminated (28°C, 48 hours)

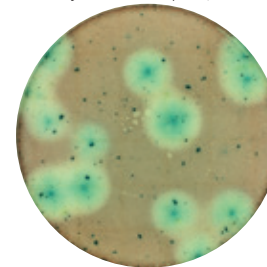


Table 2: Regression Analysis of Foods Tested on 3M™ Petrifilm™ Rapid Yeast and Mold Count Plates (48 Hours and 60 Hours)

Acidified Potato Dextrose Agar (5 Days)						
25°C	R <sup>2</sup> (48 Hours)	R <sup>2</sup> (60 Hours)	Slope (48 Hours)	Slope (60 Hours)	Intercept (48 Hours)	Intercept (60 Hours)
2015-07TD: PPE44 2X	0.904	0.959	0.995	1.014	-0.113	-0.021
42-0026-17019: PPE52 2X	0.885	0.960	0.996	1.010	-0.136	-0.003
42-0026-17027: PPE52 2X	0.915	0.964	0.992	1.007	-0.072	0.006
28°C	R <sup>2</sup> (48 Hours)	R <sup>2</sup> (60 Hours)	Slope (48 Hours)	Slope (60 Hours)	Intercept (48 Hours)	Intercept (60 Hours)
2015-07TD: PPE44 2X	0.939	0.974	1.024	1.039	-0.139	-0.084
42-0026-17019: PPE52 2X	0.932	0.974	1.025	1.037	-0.132	-0.056
42-0026-17027: PPE52 2X	0.967	0.974	1.038	1.041	-0.143	-0.091

DRBC/DG-18 (5 Days)						
25°C	R <sup>2</sup> (48 Hours)	R <sup>2</sup> (60 Hours)	Slope (48 Hours)	Slope (60 Hours)	Intercept (48 Hours)	Intercept (60 Hours)
2015-07TD: PPE44 2X	0.877	0.929	0.969	1.002	-0.124	-0.061
42-0026-17019: PPE52 2X	0.837	0.940	0.977	1.006	-0.170	-0.069
42-0026-17027: PPE52 2X	0.868	0.932	0.969	0.995	-0.088	-0.026
28°C	R <sup>2</sup> (48 Hours)	R <sup>2</sup> (60 Hours)	Slope (48 Hours)	Slope (60 Hours)	Intercept (48 Hours)	Intercept (60 Hours)
2015-07TD: PPE44 2X	0.877	0.948	0.991	1.024	-0.156	-0.135
42-0026-17019: PPE52 2X	0.884	0.950	1.001	1.030	-0.179	-0.137
42-0026-17027: PPE52 2X	0.906	0.946	1.007	1.025	-0.171	-0.141

Table 3: Comparative Log<sub>10</sub> CFU/mL Counts on Three Lots of 3M™ Petrifilm™ Rapid Yeast and Mold Count Plates (48 hours and 60 hours)

YEAST	Log <sub>10</sub> CFU/mL														25°C		Reference Counts (25°C)	
	25°C						28°C						DRBC (5 Days)	acidifiedPDA (5 Days)	DRBC (5 Days)	acidifiedPDA (5 Days)		
	2015-07TD: PPE44 (48 Hours)	2015-07TD: PPE44 (60 Hours)	42-0026-17019: PPE52 (48 Hours)	42-0026-17019: PPE52 (60 Hours)	42-0026-17027: PPE52 (48 Hours)	42-0026-17027: PPE52 (60 Hours)	2015-07TD: PPE44 (48 Hours)	2015-07TD: PPE44 (60 Hours)	42-0026-17019: PPE52 (48 Hours)	42-0026-17019: PPE52 (60 Hours)	42-0026-17027: PPE52 (48 Hours)	42-0026-17027: PPE52 (60 Hours)						
<i>Saccharomyces cerevisiae</i> (Y41)	6.33	6.34	6.28	6.28	6.27	6.28	6.22	6.20	6.25	6.22	6.19	6.25	6.22	6.23	6.22	6.23		
<i>Hansenula anomala</i> (Y28)	6.77	6.75	6.81	6.80	6.83	6.81	6.80	6.81	6.80	6.81	6.82	6.81	6.84	6.81	6.84	6.81		
<i>Candida albicans</i>	6.93	6.96	6.93	6.98	6.91	6.95	6.97	6.98	6.96	6.98	6.98	6.97	6.98	6.99	6.98	6.99		
<i>R mucaliginosa</i>	6.89	6.96	6.83	6.90	6.83	6.92	6.89	6.90	6.88	6.97	6.96	6.96	6.97	6.97	6.97	6.97		
<i>T mucoides</i>	6.45	6.46	6.61	6.62	6.60	6.62	6.53	6.56	6.48	6.54	6.56	6.49	6.41	4.78	6.41	4.78		
<i>Candida guilliermondii</i>	6.82	6.95	6.85	6.97	6.84	6.92	6.93	6.95	6.97	6.97	7.00	6.99	7.00	6.93	7.00	6.93		
<i>S lactis</i>	6.67	6.67	6.70	6.69	6.63	6.62	6.66	6.69	6.73	6.67	6.69	6.73	6.69	6.69	6.69	6.69		
<i>Candida sphaerica</i>	6.50	6.50	6.43	6.42	6.54	6.53	6.41	6.46	6.53	6.41	6.45	6.53	6.53	6.38	6.53	6.38		
<i>Candida tropicalis</i>	6.58	6.59	6.57	6.58	6.51	6.53	6.55	6.59	6.60	6.52	6.59	6.61	6.58	6.60	6.58	6.60		
<i>Kluyveromyces lactis</i> 8563	6.68	6.68	6.65	6.65	6.65	6.65	6.64	6.67	6.65	6.64	6.66	6.65	6.62	6.59	6.62	6.59		
<i>Kluyveromyces lactis</i> 10689	6.61	6.61	6.57	6.57	6.52	6.52	6.58	6.57	6.54	6.58	6.58	6.54	6.45	6.39	6.45	6.39		
<i>Debaryomyces hansenii</i>	5.70	5.71	5.72	5.73	5.75	5.75	5.60	5.69	5.68	5.77	5.79	5.75	5.81	5.56	5.81	5.56		
<i>Candida catenulata</i>	6.68	6.72	6.62	6.69	6.69	6.72	6.69	6.70	6.69	6.71	6.69	6.70	6.46	6.70	6.46	6.70		
<i>Candida glabrata</i>	6.71	6.71	6.71	6.72	6.73	6.73	6.79	6.72	6.72	6.83	6.74	6.73	6.78	6.62	6.78	6.62		
<i>Candida kefyri</i>	6.24	6.35	6.22	6.32	6.30	6.37	6.42	6.29	6.26	6.43	6.32	6.27	6.29	6.23	6.29	6.23		
<i>Candida krusei</i>	5.59	5.60	5.58	5.60	5.60	5.60	5.64	5.65	5.60	5.65	5.65	5.60	5.53	5.54	5.53	5.54		
<i>Candida lusitanae</i>	6.87	6.87	6.84	6.86	6.84	6.85	6.85	6.88	6.84	6.85	6.88	6.84	6.87	6.68	6.87	6.68		
<i>Ustilago</i> spp.	6.09	6.09	6.09	6.08	6.08	6.06	6.12	6.08	6.06	6.12	6.08	6.07	6.02	6.01	6.02	6.01		
<i>Yarrowia lipolytica</i>	6.41	6.41	6.32	6.33	6.40	6.37	6.40	6.43	6.45	6.41	6.44	6.46	6.31	6.39	6.31	6.39		

MOLD	Log <sub>10</sub> CFU/mL														25°C		Reference Counts (25°C)	
	25°C						28°C						DRBC (5 Days)	acidifiedPDA (5 Days)	DRBC (5 Days)	acidifiedPDA (5 Days)		
	2015-07TD: PPE44 (48 Hours)	2015-07TD: PPE44 (60 Hours)	42-0026-17019: PPE52 (48 Hours)	42-0026-17019: PPE52 (60 Hours)	42-0026-17027: PPE52 (48 Hours)	42-0026-17027: PPE52 (60 Hours)	2015-07TD: PPE44 (48 Hours)	2015-07TD: PPE44 (60 Hours)	42-0026-17019: PPE52 (48 Hours)	42-0026-17019: PPE52 (60 Hours)	42-0026-17027: PPE52 (48 Hours)	42-0026-17027: PPE52 (60 Hours)						
<i>Aspergillus niger</i> (M6)	4.37	4.37	4.43	4.45	4.49	4.47	4.32	4.36	4.45	4.45	4.42	4.42	4.36	4.36	4.36	4.36		
<i>Aspergillus oryzae</i>	4.34	4.51	4.32	4.50	4.35	4.46	4.48	4.51	4.55	4.44	4.45	4.44	4.39	4.24	4.39	4.24		
<i>Aspergillus brasiliensis</i>	6.26	6.35	6.20	6.31	6.28	6.40	6.33	6.33	6.34	6.36	6.36	6.38	6.32	6.33	6.32	6.33		
<i>Paecilomyces</i> (M10)	4.82	4.87	4.80	4.86	4.74	4.80	4.80	4.92	4.83	4.89	4.88	4.94	4.87	4.83	4.87	4.83		
<i>Penicillium chrysogenum</i>	6.52	6.61	6.50	6.56	6.56	6.50	6.59	6.59	6.52	6.50	6.50	6.50	6.32	6.40	6.32	6.40		
<i>Geotrichum candidum</i>	5.31	5.31	5.26	5.30	5.23	5.26	5.37	5.36	5.39	5.40	5.21	5.23	5.12	5.22	5.12	5.22		
<i>Cladosporoides</i> spp.	5.36	5.68	5.44	5.74	5.48	5.72	5.00	5.58	5.04	5.54	5.11	5.62	5.78	5.54	5.78	5.54		
<i>Scopulariopsis acremonium</i>	2.45	3.31	2.85	3.33	2.71	3.34	3.14	3.33	3.15	3.37	3.22	3.42	3.06	—	3.06	—		
<i>Mucor Racemosus</i>	5.03	5.03	4.99	4.97	4.94	4.92	4.90	4.88	4.88	4.88	4.80	4.80	5.17	4.67	5.17	4.67		
<i>Aspergillus ustus</i>	4.37	4.82	4.52	4.97	4.78	5.03	4.48	4.80	4.73	4.92	4.78	5.01	5.03	4.75	5.03	4.75		
<i>Aspergillus ustus</i>	2.74	3.49	2.63	3.53	2.88	3.59	3.38	3.69	3.27	3.59	3.37	3.64	3.64	3.19	3.64	3.19		
<i>Aspergillus flavus</i>	2.12	2.52	2.22	2.56	2.12	2.37	2.52	2.52	2.64	2.70	2.30	2.30	2.00	2.52	2.00	2.52		
<i>Aspergillus flavus</i>	6.39	6.59	6.33	6.43	6.42	6.54	6.59	6.58	6.53	6.53	6.50	6.50	6.55	—	6.55	—		
<i>Aspergillus japonicus</i>	6.41	6.60	6.40	6.55	6.47	6.54	6.58	6.59	6.59	6.59	6.54	6.54	6.56	6.55	6.56	6.55		
<i>Aspergillus aculeatus</i>	3.47	3.47	3.39	3.32	3.37	3.37	3.37	3.37	3.46	3.44	3.37	3.37	2.94	3.46	2.94	3.46		
<i>Aspergillus carbonarius</i>	5.57	5.67	5.61	5.70	5.53	5.65	5.70	5.69	5.76	5.75	5.62	5.61	5.63	5.63	5.63	5.63		
<i>Cladosporium herbarum</i>	6.80	6.91	6.74	6.90	6.79	6.69	0.00	0.00	0.00	0.00	0.00	0.00	6.79	6.82	—	—		
<i>Trichoderma virens</i>	4.82	4.87	4.75	4.85	4.70	4.85	4.88	5.90	4.75	5.90	4.92	5.85	4.99	4.80	4.99	4.80		
<i>Chaetomium globosum</i>	5.48	5.48	5.34	5.34	5.19	5.19	5.61	5.61	5.61	5.61	5.59	5.59	5.70	—	5.70	—		
<i>Geotrichum capitatum</i>	4.75	6.00	4.97	5.95	5.09	5.93	5.68	6.01	5.73	6.05	5.61	6.00	6.02	5.88	6.02	5.88		
<i>Penicillium citrinum</i>	7.02	7.01	7.05	7.08	7.08	7.06	7.06	7.08	7.04	7.06	7.08	7.03	7.08	6.95	7.08	6.95		