



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
121301

The AOAC Research Institute hereby certifies the method known as:

Petrifilm® Rapid Yeast and Mold (RYM) Count Plate

manufactured by
Neogen Corporation
620 Leshner Place
Lansing, Michigan 48912
USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

December 06, 2024
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AUTHORS ORIGINAL VALIDATION: Bob Jechorek, 3M Food Safety Department EMERGENCY RESPONSE VALIDATION MATRIX EXTENSION JUNE 2021: April Schumacher and Cari Lingle MODIFICATION JANUARY 2022: Micki L. Rosauer, Cari K. Lingle, Karen M. Silbernagel, and Jonathan Blackburn		SUBMITTING COMPANY 3M Company 3M Center, Bldg. 275-5W-05 St. Paul, MN 55144-1000 USA	CURRENT SPONSOR Neogen Food Safety Corporation 620 Leshner Place Lansing, MI 48912
METHOD NAME Neogen® Petrifilm® Rapid Yeast and Mold (RYM) Count Plate Formerly 3M™ Petrifilm™ Rapid Yeast and Mold (RYM) Count Plate		CATALOG NUMBERS Box 6475; Case 6477	
ORIGINAL VALIDATION INDEPENDENT LABORATORY Aegis Laboratories 224 N. Derby Lane North Sioux City, SD 57049	CONTRACT LABORATORY and MODIFICATION JANUARY 2022 Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204	APPLICABILITY OF METHOD Target organism – Yeasts and Molds. Matrixes – (BAM Ch. 18, ISO 21527-1:2008 Parts 1 and 2; 25 g) - yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami, frozen ground beef patties at 48 and 60 hours. ERV MATRIX EXTENSION JUNE 2021 – (10 g) Dried cannabis flower (THC >0.3%) MODIFICATION JANUARY 2022 – (BAM Ch. 18, 100 cm²) – stainless steel, sealed concrete, and rubber surfaces. Performance claims – ORIGINAL VALIDATION – These studies have demonstrated that the Petrifilm Rapid Yeast and Mold Count Plate method is a specific, sensitive, and rugged method that detects and enumerates yeast and mold at 48 and 60 hours from a variety of matrixes, yielding equivalent values to the ISO 21527:2008 parts 1 and 2 (6,7) and the FDA BAM Chapter 18 (5) reference methods. The June 2021 ERV modification demonstrated the method is equivalent to the average log counts of Dichloran Rose Bengal Chloramphenicol (DRBC) agar (8) for dried cannabis flower after 60 -72 h at 25 +/- 1 C and 28 +/- 1C. MODIFICATION JANUARY 2022 – The Petrifilm RYM Plate is equivalent to that of the FDA BAM Ch 18: <i>Yeasts, Molds and Mycotoxins</i> (5) for stainless steel, rubber, and sealed concrete environmental surfaces (100 cm²) after 48 to 60 h at 25 ± 1°C and 28 ± 1°C.	
ERV INDEPENDENT LABORATORY+ Steadfast Analytical Laboratory 21928 John R. Road Hazel Park, MI 48030	CONTRACT LABORATORY North Coast Testing Laboratories of Michigan, LLC. 1275 W. Beecher Road Adrian, MI 49211		
ORIGINAL CERTIFICATION DATE December 18, 2013		CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD - January 2019 Level 1 - June 2021 Level 2 - November 2021 Level 1 - January 2022 Level 3 - December 2022 Level 1 - January 2024 Level 1		SUMMARY OF MODIFICATION - Editorial and formatting changes of insert. - ERV matrix extension to add cannabis flower (THC >0.3%). - Editorial changes. - Matrix extension to add stainless steel, sealed concrete, and rubber surfaces. - Editorial changes. - Editorial changes to rebrand method from 3M to Neogen Corporation.	
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PRINCIPLE (1)

The Neogen® Petrifilm® Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration but does not differentiate species yeast mold from one another. Petrifilm RYM is used for the enumeration of yeast and mold in the food and beverage industries and has been validated for yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties.

DISCUSSION OF THE VALIDATION STUDY (1)

The Petrifilm Rapid Yeast and Mold Count Plate method was compared to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods at 48 and 60 hours for the detection and enumeration of yeast and mold from a variety of foods. Ten food matrixes – yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties – were inoculated with coliforms at low, medium and high levels. The log counts from the Petrifilm RYM Plate method were compared with log counts from reference methods.

Paired t-tests indicated there were no significant differences in detection or enumeration between the Petrifilm RYM Plate method and the ISO and BAM reference methods at any level at both 48 and 60 hours for yogurt, sliced apples, fermented salami, and frozen ground beef patties. For these matrixes, the Petrifilm RYM method had better repeatability with the exceptions of yogurt at the low level inoculum at both 48 and 60 hours, and the frozen ground beef patties at the high level inoculation level at both 48 and 60 hours, for which the reference methods had better repeatability.

For almonds, frozen bread dough, and ready-made pies, detection and enumeration was the same for both the Petrifilm RYM method and the reference methods at low and medium inoculation levels at 48 and 60 hours. At the high inoculation level for these matrixes, the Petrifilm RYM method had statistically better detection and enumeration at 48 and 60 hours ($p=0.004$, $0.002/0.0015$, 0.0001 respectively). The repeatability for almonds the low and high inoculation levels at both 48 and 60 hours favored the Petrifilm RYM method, but the reference methods had better repeatability at the medium inoculation level at both time points. There were no differences in recovery at the low and medium inoculation levels for sour cream and sandwiches at 48 and 60 hours. For both matrixes there was a statistically significant difference at the high inoculation level ($p=0.0457$ and $p=0.029$ respectively) which favored the reference methods. For sour cream at the low and medium inoculation levels at both time points, the Petrifilm RYM method had better repeatability. The reference methods had better repeatability at the high inoculation level. For sandwiches, the Petrifilm RYM method had better repeatability at the high inoculation level at both 48 and 60 hours, whereas the reference methods had better repeatability at the low and medium inoculation levels at both time points.

There was no recovery of the inoculated organism (*Paecilomyces* spp) on the reference medium (DG-18) from dehydrated soup. Five lots of dehydrated soup, with increasing inoculation levels, were tested in an attempt to gain recovery of the target organism recovery on DG-18. In addition, pre-poured DG-18 agar medium was utilized instead of the dehydrated, laboratory made medium as another attempt to recover the target organism. The General Referee was contacted for advice. The decision was made that since the *Paecilomyces* spp was not recovered with attempts to increase the inoculum titer that the data would be accepted with no recovery from the DG-18 reference medium. There is no explanation for why the DG-18 reference medium does not recover the organism from dehydrated soup. The organism does grow on the medium when grown in pure culture.

In strain studies, of the 19 yeast and 23 mold strains that should have been detected by the Petrifilm RYM Plate method, all were detected and had appropriate colonial morphology, giving a sensitivity of 100%. Of the 4 non-target organisms that should not been detected the Petrifilm RYM Plate method, none of the 4 strains were detected, yielding a specificity of 100%.

Table 9. Inclusivity Organisms (1)

Organism-Yeast- 48 hours	Source
Y41: <i>Saccharomyces cerevisiae</i>	Neogen culture collection
Y28: <i>Hansenula anomala</i>	Neogen culture collection
<i>Candida albicans</i>	Neogen culture collection
<i>Rh. Mucaliginosa</i>	Microbiologics
<i>Trichosporon mucoides</i>	Microbiologics
<i>Candida guilliermondii</i>	Microbiologics
<i>Saccharomyces lactis</i>	Microbiologics
<i>Candida sphaerica</i>	Microbiologics
<i>Candida tropicalis</i>	Microbiologics
<i>Kluyveromyces lactis</i> ATCC 8563	Microbiologics
<i>Kluyveromyces lactis</i> ATCC 10689	Microbiologics
<i>Debaryomyces hansenii</i>	Microbiologics
<i>Candida catenulata</i> ATCC 10565	Microbiologics
<i>Candida glabrata</i>	Microbiologics
<i>Candida kefyr</i> ATCC 204093	Microbiologics
<i>Candida krusei</i> ATCC 14243	Microbiologics
<i>Candida lusitanae</i> ATCC 34449	Microbiologics
<i>Ustilago</i> spp.	Microbiologics
<i>Yarrowia lipolytica</i>	Neogen culture collection

Organism-Molds- 48 hours	Source
<i>Aspergillus niger</i> (M6) (re- classified as <i>brasiliensis</i>)	Microbiologics
<i>Aspergillus oryzae</i>	Microbiologics
<i>Aspergillus brasiliensis</i>	Microbiologics
<i>Paecilomyces</i> sp (M10)	Microbiologics
<i>Penicillium chrysogenum</i>	Microbiologics
<i>Geotrichum candidum</i>	Microbiologics
<i>Cladosporium</i> spp	Microbiologics
<i>Scorulisporium acremonium</i>	Microbiologics
<i>Mucor racemosus</i>	Microbiologics/NCPF
<i>Aspergillus ustus</i>	Microbiologics
<i>Aspergillus flavus</i>	Neogen culture collection
<i>Aspergillus japonicus</i>	Neogen culture collection
<i>Aspergillus aculeatus</i>	Neogen culture collection
<i>Aspergillus carbonarius</i>	Neogen culture collection
<i>Cladosporium herbarum</i>	Neogen culture collection
<i>Trichoderma virens</i>	Neogen culture collection
<i>Chaetomium globosum</i>	Microbiologics
<i>Geotrichum capitatum</i>	Microbiologics
Yeasts and Molds- Slow growing- 60 hrs	Source
<i>Zygosaccharomyces rouxii</i> ^a	Microbiologics
<i>Zygosaccharomyces bailii</i> ^a	Microbiologics
<i>Penicillium aurantiogriseum</i> ^b	Microbiologics
<i>Aspergillus fumigatus</i> ^c	Microbiologics
<i>Botrytis</i> sp (M97) ^c	Microbiologics
^a <i>Z. bailii</i> and <i>Z. rouxii</i> are slow growing yeasts - require 60 hours at 25 or 28C	
^b <i>P. aurantiogriseum</i> is a slow growing mold - requires 60 hours at 25 or 28C	
^c : <i>A. fumigatus</i> and M97 prefer a higher temperature for growth. These molds are visible on the Rapid Yeast and Mold plates within 48hrs at 28C but require 60 hours to grow at 25C	
All 42 organisms tested on the Petrifilm RYM Count Plate were detected as typical colonies, yielding a sensitivity of 100%.	

Table 10: Exclusivity Organisms (1)

Organism	Source
<i>Escherichia coli</i> ATCC 25922	Neogen culture collection
<i>Bacillus subtilis</i> ATCC 6633	Neogen culture collection
<i>Aeromonas hydrophila</i> ATCC 7965	Neogen culture collection
<i>Enterococcus faecalis</i> ATCC 14506	Neogen culture collection

Table 11: Method Comparison Results for Yogurt at 48 Hours (1)

Level	Sample	3M RYM Petrifilm				ISO/ FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0			<10	<0			NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			-	-			
Low	1	2.8 x10 ²	2.4472			2.6x10 ²	2.415			0.6343
	2	2.0 x10 ²	2.301			3.2 x10 ²	2.5051			
	3	3.0x10 ²	2.4771			3.0 x10 ²	2.4771			
	4	3.2x10 ²	2.5051			3.2 x10 ²	2.5051			
	5	3.8x10 ²	2.5798			2.462	0.1026			
Medium	1	2.8 x10 ³	3.4472			2.4x10 ³	3.3802			0.4633
	2	2.4 x10 ³	3.3802			3.6x10 ³	3.5563			
	3	2.8 x10 ³	3.4472			2.6 x10 ³	3.415			
	4	2.6 x10 ³	3.415			3.4 x10 ³	3.5315			
	5	2.6 x10 ³	3.415			3.4209	0.0279			
High	1	2.0 x 10 ⁴	4.301			2.2 x 10 ⁴	4.3424			0.6776
	2	1.6 x 10 ⁴	4.2041			1.6 x 10 ⁴	4.2041			
	3	2.0 x 10 ⁴	4.301			2.2 x 10 ⁴	4.3424			
	4	1.6 x 10 ⁴	4.2041			1.4 x 10 ⁴	4.1461			
	5	1.3 x 10 ⁴	4.1139			4.2248	0.0787			

Table 12: Method Comparison Results for Yogurt at 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO / FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	2.8 x10 ²	2.4472	2.4458	0.0942	2.6 x10 ²	2.415	2.4868	0.0445	0.3973
	2	2.0 x10 ²	2.301			3.2 x 10 ²	2.5051			
	3	2.8 x10 ²	2.4472			3.0 x1 0 ²	2.4771			
	4	3.0 x 10 ²	2.4771			3.2 x 10 ²	2.5051			
	5	3.6 x 10 ²	2.5563			3.4 x 10 ²	2.5315			
Medium	1	2.8 x10 ³	3.4472	3.4209	0.0279	2.4 x10 ³	3.3802	3.4526	0.085	0.4633
	2	2.4 x10 ³	3.3802			3.6 x10 ³	3.5563			
	3	2.8 x10 ³	3.4472			2.6 x10 ³	3.415			
	4	2.6 x10 ³	3.415			3.4 x10 ³	3.5315			
	5	2.6 x10 ³	3.415			2.4 x10 ³	3.3802			
High	1	2.0 x 10 ⁴	4.301	4.2248	0.0787	2.2 x 10 ⁴	4.3424	4.2478	0.0895	0.6776
	2	1.6 x 10 ⁴	4.2041			1.6 x 10 ⁴	4.2041			
	3	2.0 x 10 ⁴	4.301			2.2 x 10 ⁴	4.3424			
	4	1.6 x 10 ⁴	4.2041			1.4 x 10 ⁴	4.1461			
	5	1.3 x 10 ⁴	4.1139			1.6 x 10 ⁴	4.2041			

Table 13: Method Comparison Results for Sour Cream at 48 and 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	8.2x10 ¹	1.9138	1.243	0.397	9.2 x 10 ¹	1.9638	1.1928	0.431	0.8528
	2	1.0x10 ¹	1.0000			1.0 x 10 ¹	1.0000			
	3	1.0x10 ¹	1.0000			1.0 x 10 ¹	1.0000			
	4	1.0x10 ¹	1.0000			1.0 x 10 ¹	1.0000			
	5	2.0x10 ¹	1.301			1.0x 10 ¹	1.0000			
Medium	1	1.0x10 ²	2.0000	1.9095	0.0681	5.4 x 10 ³	1.8451	1.959	0.0733	0.3007
	2	7.0x10 ¹	1.8451			2.7 x 10 ³	2.0000			
	3	8.0x10 ¹	1.9031			3.2 x 10 ³	1.9542			
	4	9.0x10 ¹	1.9542			5.6 x 10 ³	2.0414			
	5	7.0x10 ¹	1.8451			4.0 x 10 ³	1.9542			
High	1	1.2 x 10 ³	3.0792	2.9943	0.105	3.9 x 10 ⁴	3.1761	3.1259	0.0367	0.0457
	2	7.5 x 10 ²	2.8751			2.6 x 10 ⁴	3.0792			
	3	1.0 x 10 ³	3.0000			4.2 x 10 ⁴	3.1139			
	4	8.0 x 10 ²	2.9031			3.8 x 10 ⁴	3.1461			
	5	1.3 x 10 ³	3.1139			3.4 x 10 ⁴	3.1139			

Table 14: Method Comparison Results for Almonds at 48 and 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0			<10	<0			NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	2.0x10 ¹	1.301			2.0x10 ¹	1.301			0.1471
	2	2.0x10 ¹	1.301			1.0x10 ¹	1.0000			
	3	3.0x10 ¹	1.4771			3.0x10 ¹	1.4771			
	4	2.0x10 ¹	1.301			1.0x10 ¹	1.0000			
	5	2.0x10 ¹	1.301			2.0x10 ¹	1.0000			
Medium	1	1.4x10 ²	2.1461			1.0 x 10 ³	2.301			0.0661
	2	1.4 x10 ²	2.1461			1.4 x 10 ³	2.301			
	3	2.3 x10 ²	2.3617			1.3 x 10 ³	2.415			
	4	1.6 x10 ²	2.2041			1.0 x 10 ³	2.3222			
	5	1.7x10 ²	2.2304			2.3 x 10 ³	2.2553			
High	1	6.2 x 10 ²	2.7924			5.0 x 10 ⁴	2.699			0.0045
	2	6.8 x 10 ²	2.8325			6.2 x 10 ⁴	2.6435			
	3	6.0 x 10 ²	2.7782			5.8 x 10 ⁴	2.6335			
	4	6.2 x 10 ²	2.7924			4.9 x 10 ⁴	2.6902			
	5	7.2 x 10 ²	2.8573			5.3 x 10 ⁴	2.7853			

Table 15: Method Comparison Results for Sliced Apples at 48 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Low	1	4.0 x10 ¹	1.6021			4.0x10 ¹	1.6021			
	2	1.0 x10 ¹	1.0000			1.0 x10 ¹	1.0000			
	3	1.0x10 ¹	1.0000			1.0 x10 ¹	1.0000			
	4	2.0x10 ¹	1.301			<10	<0			
	5	2.0x10 ¹	1.301			<10	<0			
Medium	1	4.4 x10 ²	2.6435			4.6x10 ²	2.6628			
	2	1.2 x10 ²	2.0792			1.1x10 ²	2.0414			
	3	1.4 x10 ²	2.1461			1.1 x10 ²	2.0414			
	4	1.2 x10 ²	2.0792			4.0 x10 ¹	1.6021			
	5	1.6 x10 ²	2.2041			1.5 x10 ²	2.1761			
High	1	1.8 x 10 ⁴	4.2553			3.0 x 10 ⁴	4.4771			
	2	1.6 x 10 ⁴	4.2041			1.9 x 10 ⁴	4.2788			
	3	2.5 x 10 ⁴	4.3979			3.6 x 10 ⁴	4.5563			
	4	2.6 x 10 ⁴	4.415			4.1x 10 ⁴	4.6128			
	5	1.4 x 10 ⁴	4.1461			1.9 x 10 ⁴	4.2788			

Table 16: Method Comparison Results for Sliced Apples at 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Low	1	4.0 x10 ¹	1.6021	1.2408	0.2519	4.0x10 ¹	1.6021	1.2007	0.3476	0.8546
	2	1.0 x10 ¹	1.0000			1.0 x10 ¹	1.0000			
	3	1.0x10 ¹	1.0000			1.0 x10 ¹	1.0000			
	4	2.0x10 ¹	1.301			<10	<0			
	5	2.0x10 ¹	1.301			<10	<0			
Medium	1	4.4 x10 ²	2.6435	2.2908	0.2133	4.6x10 ²	2.6628	2.4414	0.3799	0.3675
	2	1.5 x10 ²	2.1761			1.1x10 ²	2.0414			
	3	1.4 x10 ²	2.1461			1.1 x10 ²	2.0414			
	4	1.4 x10 ²	2.1461			4.0 x10 ¹	1.6021			
	5	2.2x10 ²	2.3424			1.5 x10 ²	2.1761			
High	1	1.8 x 10 ⁴	4.2553	4.2837	0.1187	3.0 x 10 ⁴	4.4771	4.4407	0.1555	0.1104
	2	1.6 x 10 ⁴	4.2041			1.9 x 10 ⁴	4.2788			
	3	2.5 x 10 ⁴	4.3979			3.6 x 10 ⁴	4.5563			
	4	2.6 x 10 ⁴	4.415			4.1x 10 ⁴	4.6128			
	5	1.4 x 10 ⁴	4.1461			1.9 x 10 ⁴	4.2788			

Table 17. Method Comparison Results for frozen Bread Dough at 48 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Low	1	2.2 x10 ²	2.3424			2.1 x10 ²	2.3222			
	2	1.8 x10 ²	2.2553			2.1 x10 ²	2.3222			
	3	2.1 x10 ²	2.3222			1.9 x10 ²	2.2788			
	4	2.0x10 ²	2.301			2.3 x10 ²	2.3617			
	5	3.5 x10 ²	2.5441			3.2 x10 ²	2.5051			
Medium	1	2.8 x10 ³	3.4472			2.0 x10 ³	3.301			
	2	2.4 x10 ³	3.3802			2.3 x10 ³	3.3617			
	3	2.6 x10 ³	3.415			2.2 x10 ³	3.3424			
	4	2.4 x10 ³	3.3802			9.4 x10 ²	2.9731			
	5	3.4 x10 ³	3.5315			3.5 x10 ³	3.5441			
High	1	2.5 x 10 ⁴	4.3979			1.8 x 10 ⁴	4.2553			
	2	2.2 x 10 ⁴	4.3424			8.0 x 10 ³	3.9031			
	3	2.1 x 10 ⁴	4.3222			8.6 x 10 ³	3.9345			
	4	1.8 x 10 ⁴	4.2553			6.5 x 10 ³	3.8129			
	5	3.6 x 10 ⁴	4.5563			8.3 x 10 ³	3.9191			

Table 18: Method Comparison Results for frozen Bread Dough at 60 Hours.(1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM			p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	MEAN	SD (S _r)	
Low	1	2.2 x10 ²	2.3424	2.3836	0.1054	2.1 x10 ²	2.358	0.0873	0.6873
	2	1.9 x10 ²	2.2788			2.1 x10 ²			
	3	2.5 x10 ²	2.3979			1.9 x10 ²			
	4	2.2 x10 ²	2.3424			2.3 x10 ²			
	5	3.6 x10 ²	2.5563			3.2 x10 ²			
Medium	1	2.9 x10 ³	3.4624	3.4374	0.0608	2.0 x10 ³	3.3045	0.2073	0.2274
	2	2.5 x10 ³	3.3979			2.3 x10 ³			
	3	2.6 x10 ³	3.415			2.2 x10 ³			
	4	2.4 x10 ³	3.3802			9.4 x10 ²			
	5	3.4 x10 ³	3.5315			3.5 x10 ³			
High	1	2.6 x 10 ⁴	4.415	4.3987	0.1154	1.8 x 10 ⁴	3.965	0.169	0.0015
	2	2.5 x 10 ⁴	4.3979			8.0 x 10 ³			
	3	2.1 x 10 ⁴	4.3222			8.6 x 10 ³			
	4	1.9 x 10 ⁴	4.2788			6.5 x 10 ³			
	5	3.8 x 10 ⁴	4.5798			8.3 x 10 ³			

Table 19: Method Comparison Results for Ready-made Pie at 48 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	2.0x10 ¹	1.301	1.1204	0.1649	1.0x10 ¹	1.0000	1.2007	0.1738	0.537
	2	1.0x10 ¹	1.0000			2.0x10 ¹	1.301			
	3	1.0x10 ¹	1.0000			2.0x10 ¹	1.301			
	4	1.0x10 ¹	1.0000			<10	<0			
	5	2.0x10 ¹	1.301			<10	<0			
Medium	1	1.2x10 ²	2.0792	2.2049	0.1467	1.0 x 10 ³	2.0000	2.1244	0.1482	0.4128
	2	1.4 x10 ²	2.1461			1.4 x 10 ³	2.1461			
	3	1.2 x10 ²	2.0792			1.3 x 10 ³	2.1139			
	4	2.1x10 ²	2.3222			1.0 x 10 ³	2.0000			
	5	2.5x10 ²	2.3979			2.3 x 10 ³	2.3617			
High	1	7.2 x 10 ³	3.8573	3.8944	0.0324	5.0 x 10 ⁴	3.699	3.7339	0.0433	0.0001
	2	7.7 x 10 ²	3.8865			6.2 x 10 ⁴	3.7924			
	3	8.0 x 10 ³	3.9031			5.8 x 10 ⁴	3.7634			
	4	7.6 x 10 ²	3.8808			4.9 x 10 ⁴	3.6902			
	5	8.8 x 10 ³	3.9445			5.3 x 10 ⁴	3.7243			

Table 20: Method Comparison Results for Ready-made Pie at 60 Hours.(1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	2.0x10 ¹	1.301	1.1204	0.1649	1.0x10 ¹	1	1.2007	0.1738	0.537
	2	1.0x10 ¹	1			2.0x10 ¹	1.301			
	3	1.0x10 ¹	1			2.0x10 ¹	1.301			
	4	1.0x10 ¹	1			<10	<0			
	5	2.0x10 ¹	1.301			<10	<0			
Medium	1	1.3x10 ²	2.1139	2.2119	0.1399	1.0 x 10 ³	2	2.1244	0.1482	0.3651
	2	1.4 x10 ²	2.1461			1.4 x 10 ³	2.1461			
	3	1.2 x10 ²	2.0792			1.3 x 10 ³	2.1139			
	4	2.1x10 ²	2.3222			1.0 x 10 ³	2			
	5	2.5x10 ²	2.3979			2.3 x 10 ³	2.3617			
High	1	7.4 x 10 ³	3.8692	3.8991	0.0282	5.0 x 10 ⁴	3.699	3.7339	0.0433	0.0001
	2	7.8 x 10 ²	3.8921			6.2 x 10 ⁴	3.7924			
	3	8.0 x 10 ³	3.9031			5.8 x 10 ⁴	3.7634			
	4	7.7 x 10 ²	3.8865			4.9 x 10 ⁴	3.6902			
	5	8.8 x 10 ³	3.9445			5.3 x 10 ⁴	3.7243			

Table 21: Method Comparison Results for Sandwiches at 48 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Low	1	2.0 x10 ¹	1.301	1.4214	0.2692	4.0x10 ¹	1.6021	1.3362	0.227	0.6033
	2	4.0 x10 ¹	1.6021			3.0 x10 ¹	1.4771			
	3	4.0x10 ¹	1.6021			2.0 x10 ¹	1.301			
	4	4.0x10 ¹	1.6021			2.0 x10 ¹	1.301			
	5	1.0x10 ¹	1.0000			1.0x10 ¹	1.0000			
Medium	1	3.6 x10 ²	2.5563	2.3872	0.1535	2.7x10 ²	2.4314	2.4414	0.0522	0.4879
	2	3.2 x10 ²	2.5051			2.7x10 ²	2.4314			
	3	1.6 x10 ²	2.2041			2.5 x10 ²	2.3979			
	4	2.6 x10 ²	2.415			2.6 x10 ²	2.415			
	5	1.8 x10 ²	2.2553			3.4 x10 ²	2.5315			
High	1	1.1 x 10 ⁴	4.0414	4.0055	0.0494	1.1 x 10 ⁴	4.0414	4.2021	0.1363	0.029
	2	1.1 x 10 ⁴	4.0414			1.4 x 10 ⁴	4.1461			
	3	9.1 x 10 ³	3.959			2.6 x 10 ⁴	4.415			
	4	1.1 x 10 ⁴	4.0414			1.6 x 10 ⁴	4.2041			
	5	8.8 x 10 ³	3.9445			1.6 x 10 ⁴	4.2041			

Table 22: Method Comparison Results for Sandwiches at 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Low	1	2.0 x10 ¹	1.301	1.4214	0.2692	4.0x10 ¹	1.6021	1.3362	0.227	0.6033
	2	4.0 x10 ¹	1.6021			3.0 x10 ¹	1.4771			
	3	4.0x10 ¹	1.6021			2.0 x10 ¹	1.301			
	4	4.0x10 ¹	1.6021			2.0 x10 ¹	1.301			
	5	1.0x10 ¹	1.0000			1.0x10 ¹	1.0000			
Medium	1	3.6 x10 ²	2.5563	2.3872	0.1535	2.7x10 ²	2.4314	2.4414	0.0522	0.4879
	2	3.2 x10 ²	2.5051			2.7x10 ²	2.4314			
	3	1.6 x10 ²	2.2041			2.5 x10 ²	2.3979			
	4	2.6 x10 ²	2.415			2.6 x10 ²	2.415			
	5	1.8 x10 ²	2.2553			3.4 x10 ²	2.5315			
High	1	1.1 x 10 ⁴	4.0414	4.0131	0.0396	1.1 x 10 ⁴	4.0414	4.2021	0.1363	0.0308
	2	1.1 x 10 ⁴	4.0414			1.4 x 10 ⁴	4.1461			
	3	9.6 x 10 ³	3.9823			2.6 x 10 ⁴	4.415			
	4	1.1 x 10 ⁴	4.0414			1.6 x 10 ⁴	4.2041			
	5	9.1 x 10 ³	3.959			1.6 x 10 ⁴	4.2041			

Table 23: Method Comparison Results for Dehydrated Soup at 48 and 60 Hours.(1)

Table 23: Method comparison Results for Dehydrated Soup at 48 and 60 Hours.(1)										
Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Low	1	2.0 x10 ¹	1.301	1.276	0.1721	<10	<0	-	-	NA
	2	3.0 x10 ¹	1.4771			<10	<0			
	3	1.0x10 ¹	1.0000			<10	<0			
	4	2.0x10 ¹	1.301			<10	<0			
	5	2.0x10 ¹	1.301			<10	<0			
Medium	1	3.1 x10 ²	2.4914	2.4234	0.1132	<10	<0	-	-	NA
	2	3.4 x10 ²	2.5315			<10	<0			
	3	2.3 x10 ²	2.3617			<10	<0			
	4	3.0 x10 ²	2.4771			<10	<0			
	5	1.8 x10 ²	2.2553			<10	<0			
High	1	1.3 x 10 ⁴	3.1139	2.9145	0.1366	<10	<0	-	-	NA
	2	7.2 x 10 ⁴	2.8573			<10	<0			
	3	9.9 x 10 ⁴	2.9956			<10	<0			
	4	6.4 x 10 ⁴	2.8062			<10	<0			
	5	6.3 x 10 ⁴	2.7993			<10	<0			

Table 24: Method Comparison Results for Fermented Salami at 48 Hours.(1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	<10	<0	1.3637	0.4671	<10	<0	1.4758	0.4273	0.7354
	2	9.5 x10 ¹	1.9777			1.0x10 ²	2.0000			
	3	1.0x10 ¹	1.0000			2.0 x10 ¹	1.3010			
	4	1.0x10 ¹	1.0000			1.0 x10 ¹	1.0000			
	5	3.0x10 ¹	1.4771			4.0x10 ¹	1.6021			
Medium	1	3.2 x10 ²	2.5051	2.5089	0.0686	3.4x10 ²	2.5315	2.4942	0.0554	0.7194
	2	3.6 x10 ²	2.5563			3.4x10 ²	2.5315			
	3	3.9 x10 ²	2.5911			3.2 x10 ²	2.5051			
	4	3.0 x10 ²	2.4771			2.5 x10 ²	2.3979			
	5	2.6 x10 ²	2.415			3.2x10 ²	2.5051			
High	1	2.5 x 10 ³	3.3979	3.4035	0.0385	3.5 x 10 ⁴	3.3010	3.3568	0.0352	0.0805
	2	2.6 x 10 ³	3.4150			4.3 x 10 ⁴	3.3802			
	3	2.6 x 10 ³	3.4150			4.9 x 10 ⁴	3.3802			
	4	2.2 x 10 ³	3.3424			4.5x 10 ⁴	3.3424			
	5	2.8 x 10 ³	3.4472			5.2 x 10 ⁴	3.3802			

Table 25: Method Comparison Results for Fermented Salami at 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	<10	<0	1.4109	0.5072	<10	<0	1.4758	0.4273	0.1461
	2	1.1 x10 ²	2.0414			1.0x10 ²	2.0000			
	3	1.0x10 ¹	1.0000			2.0 x10 ¹	1.3010			
	4	1.0x10 ¹	1.0000			1.0 x10 ¹	1.0000			
	5	4.0x10 ¹	1.6021			4.0x10 ¹	1.6021			
Medium	1	3.2 x10 ²	2.5051	2.5089	0.0686	3.4x10 ²	2.5315	2.4942	0.0554	0.7194
	2	3.6 x10 ²	2.5563			3.4x10 ²	2.5315			
	3	3.9 x10 ²	2.5911			3.2 x10 ²	2.5051			
	4	3.0 x10 ²	2.4771			2.5 x10 ²	2.3979			
	5	2.6 x10 ²	2.415			3.2x10 ²	2.5051			
High	1	2.5 x 10 ³	3.3979	3.4095	0.0481	3.5 x 10 ⁴	3.301	3.3568	0.0352	0.0837
	2	2.6 x 10 ³	3.415			4.3 x 10 ⁴	3.3802			
	3	2.6 x 10 ³	3.415			4.9 x 10 ⁴	3.3802			
	4	2.2 x 10 ³	3.3424			4.5x 10 ⁴	3.3424			
	5	3.0 x 10 ³	3.4771			5.2 x 10 ⁴	3.3802			

Table 26: Method Comparison Results for Frozen Ground Beef Patties at 48 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0	-	-	<10	<0	-	-	NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			<10	<0			
Low	1	4.0x10 ¹	1.6021	1.6373	0.227	5.0 x10 ¹	1.699	1.7204	0.1104	0.7482
	2	2.0x10 ¹	1.301			5.0x10 ¹	1.699			
	3	4.0x10 ¹	1.6021			5.0 x10 ¹	1.699			
	4	6.0x10 ¹	1.7782			4.0 x10 ¹	1.6021			
	5	8.0x10 ¹	1.9031			8.0x10 ¹	1.9031			
Medium	1	1.9x10 ²	2.2788	2.3468	0.1215	2.2x10 ²	2.3424	2.3722	0.139	0.7657
	2	2.2 x10 ²	2.3424			2.3x10 ²	2.3617			
	3	2.0 x10 ²	2.301			1.6 x10 ²	2.2041			
	4	1.8 x10 ²	2.2553			3.9 x10 ²	2.5911			
	5	3.6x10 ²	2.5563			2.3x10 ²	2.3617			
High	1	5.0 x 10 ³	3.699	3.6877	0.0276	3.5 x 10 ⁴	3.5441	3.6288	0.0538	0.0613
	2	4.4 x 10 ³	3.6435			4.3 x 10 ⁴	3.6335			
	3	5.2 x 10 ³	3.716			4.9 x 10 ⁴	3.6902			
	4	5.0 x 10 ³	3.699			4.5x 10 ⁴	3.6532			
	5	4.8 x 10 ³	3.6812			5.2 x 10 ⁴	3.6232			

Table 27: Method Comparison Results for Frozen Ground Beef Patties at 60 Hours. (1)

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S _r)	Ct/g	LOG	MEAN	SD (S _r)	
Uninoculated	1	<10	<0			<10	<0			NA
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0			-	-			
Low	1	4.0x10 ¹	1.6021			5.0 x10 ¹	1.699			0.7542
	2	3.0x10 ¹	1.4771			5.0x10 ¹	1.699			
	3	5.0x10 ¹	1.699			5.0 x10 ¹	1.699			
	4	6.0x10 ¹	1.7782			4.0 x10 ¹	1.6021			
	5	8.0x10 ¹	1.9031			1.6919	0.163			
Medium	1	2.0 x10 ²	2.301			2.2x10 ²	2.3424			0.933
	2	2.2 x10 ²	2.3424			2.3x10 ²	2.3617			
	3	2.0 x10 ²	2.301			1.6 x10 ²	2.2041			
	4	2.0 x10 ²	2.301			3.9 x10 ²	2.5911			
	5	3.8x10 ²	2.5798			2.3451	0.1214			
High	1	5.0 x 10 ³	3.699			3.5 x 10 ⁴	3.5441			0.0613
	2	4.4 x 10 ³	3.6435			4.3 x 10 ⁴	3.6335			
	3	5.2 x 10 ³	3.716			4.9 x 10 ⁴	3.6902			
	4	5.0 x 10 ³	3.699			4.5x 10 ⁴	3.6532			
	5	4.8 x 10 ³	3.6812			3.6877	0.0276			

DISCUSSION OF ERV MODIFICATION APPROVED JUNE 2021 (3)

The Petrifilm Rapid Yeast and Mold Count Plate method was incubated at 25 ± 1°C and 28 ± 1°C and compared to AOAC SMPR 2021.009, 72 hours for the detection and enumeration of yeast and mold from dried cannabis flower. Naturally contaminated cannabis flower samples were tested at low, medium and high levels. The log counts from the Petrifilm Rapid Yeast and Mold Count Plate method were compared with log counts from DRBC agar.

The 90% and 95% confidence intervals indicated there were no significant differences in detection or enumeration between the Petrifilm Rapid Yeast and Mold Count Plate method and the DRBC agar at the medium and high contamination levels at both temperatures, 25 ± 1°C and 28 ± 1°C, at 72 h. The 90% confidence interval was just outside the acceptance criterion for statistical equivalence at the low level at both temperatures, 25 ± 1°C and 28 ± 1°C at 72 h (-0.577, -0.236 and -0.682, -0.322 respectively). A higher standard deviation was observed on the agar plates (s_r > 0.2), which can lead to wider confidence intervals.

In the strain studies, of the 70 yeast and mold strains that should have been detected by the Petrifilm Rapid Yeast and Mold Count Plate method, all were detected and had appropriate colony morphology, giving a sensitivity of 100%. None of the 32 non-target strains were detected by the Petrifilm Rapid Yeast and Mold Count plate method, giving a specificity of 100%.

Table 2. Inclusivity Results for the Petrifilm RYM Plates (3)

No.	Organism	Source	Origin	25°C	25°C	28°C	28°C
				48 h Results	60 h Results	48 h Results	60 h Results
1	<i>Alternaria alternata</i> ^a	ATCC ^b 66981	<i>Arachis hypogaea</i>	+ ^c	+	+	+
2	<i>Arthrinium species (aureum)</i> ^a	ATCC 56042	Not Available	+	+	+	+
3	<i>Aspergillus aculeatus</i>	ATCC 56925	Grape	+	+	+	+
4	<i>Aspergillus brasiliensis</i> ^{a,d}	ATCC 16404	Blueberry	+	+	+	+
5	<i>Aspergillus caesiellus</i> ^a	ATCC 42693	Dried Chilies	+	+	+	+
6	<i>Aspergillus carbonarius</i>	ATCC 6276	Not Available	+	+	+	+
7	<i>Aspergillus flavus</i>	ATCC 9643	Shoe sole	+	+	+	+
8	<i>Aspergillus fumigatus</i> ^f	ATCC 204305	Human sputum	- ^c	+	+	+
9	<i>Aspergillus japonicus</i>	ATCC 52036	Soil	+	+	+	+
10	<i>Aspergillus niger</i> ^a	ATCC 6275	Not Available	+	+	+	+
11	<i>Aspergillus niger</i> (re-classified as <i>brasiliensis</i>)	Neogen ⁱ M6	Not Available	+	+	+	+
12	<i>Aspergillus oryzae</i>	ATCC 10124	Not Available	+	+	+	+
13	<i>Aspergillus terreus</i> ^a	ATCC 1012	Soil	+	+	+	+
14	<i>Aspergillus ustus</i> (re-classified as <i>Aspergillus puniceus</i>)	ATCC 10760	Greenhouse soil	+	+	+	+
15	<i>Aureobasidium species (pullulans)</i> ^a	ATCC 15233	Painted wood	+	+	+	+
16	<i>Botrytis species</i> ^f	Neogen M97	Not Available	-	+	+	+
17	<i>Candida albicans</i>	ATCC 10231	Not Available	+	+	+	+
18	<i>Candida catenulata</i> (re-classified as <i>Diutina catenulata</i>)	ATCC 10565	Human feces	+	+	+	+
19	<i>Candida glabrata</i>	ATCC 15126	Not Available	+	+	+	+
20	<i>Candida guilliermondii</i> (re-classified as <i>Meyerozyma guilliermondii</i>)	ATCC 6260	Not Available	+	+	+	+
21	<i>Candida kefyr</i>	ATCC 204093	Not Available	+	+	+	+
22	<i>Candida krusei</i>	ATCC 14243	Not Available	+	+	+	+
23	<i>Candida lusitanae</i>	ATCC 34449	Pig	+	+	+	+
24	<i>Candida sphaerica</i>	Microbiologics ^e	Not Available	+	+	+	+
25	<i>Candida tropicalis</i>	ATCC 13803	Not Available	+	+	+	+
26	<i>Chaetomium globosum</i> ^{a,d}	ATCC 6205	Stored Cotton	+	+	+	+
27	<i>Cladosporium cladosporioides</i> ^a	Ad1405 ^h	Bakery	+	+	+	+
28	<i>Cladosporium herbarum</i> (re-classified as <i>Cladosporium macrocarpum</i>)	ATCC 76226	Sugar beet leaf	+	+	+	+
29	<i>Cladosporium spp cladosporioides</i>	ATCC 16022	painted floor	+	+	+	+
30	<i>Cryptococcus liquefaciens</i> ^a	Adria ^h 1041	Environment	+	+	+	+
31	<i>Cryptococcus magnus</i> ^a	Adria 1040	Environment	+	+	+	+
32	<i>Curvularia lunata</i> ^a	ATCC 12017	Not Available	+	+	+	+
33	<i>Trichosporon mucoides</i> (re-classified as <i>Cutaneotrichosporon dermatis</i>)	ATCC 201382	Not Available	+	+	+	+
34	<i>Debaryomyces hansenii</i>	Microbiologics	Cheese and Milk	+	+	+	+
35	<i>Fusarium oxysporum</i> ^a	QL ⁱ 0567126A	Environmental Isolate	+	+	+	+
36	<i>Fusarium proliferatum</i> ^a	QL 0567112.1C	Environmental Isolate	+	+	+	+
37	<i>Fusarium solani</i> ^g	QL 345317.4B	Environmental Isolate	+	+	+	+
38	<i>Geotrichum candidum</i> ^a	ATCC 204307	Dairy	+	+	+	+
39	<i>Geotrichum candidum</i> (re-classified as <i>Galactomyces candidus</i>)	ATCC 34614	clotted carrot	+	+	+	+
40	<i>Geotrichum capitatum</i>	Microbiologics 0482L	Not Available	+	+	+	+
41	<i>Hanseniaspora uvarum</i> ^a	CLIB ^h 303	Oenology	+	+	+	+
42	<i>Hansenula anomala</i>	Neogen Y28	N/A	+	+	+	+
43	<i>Kluyveromyces lactis</i>	ATCC 8563	Creamery	+	+	+	+
44	<i>Kluyveromyces lactis</i>	ATCC 10689	Cheese	+	+	+	+
45	<i>Kluyveromyces marxianus</i> ^a	CLIB 720	Dairy	+	+	+	+
46	<i>Mucor plumbeus</i> ^a	Adria M10	Food	+	+	+	+
47	<i>Mucor racemosus</i>	NCPF 7650	Not Available	+	+	+	+
48	<i>Paecilomyces sp (M10)</i>	Microbiologics 0287E4	Not Available	+	+	+	+
49	<i>Paecilomyces variotii</i> ^g	Adria M6	Food	-	+72 hr ^k	-	+
50	<i>Penicillium aurantiogriseum</i> (re-classified as <i>Penicillium venetum</i>)	ATCC 16025	<i>Hyacinthus</i> sp. bulb	-	+	-	+
51	<i>Penicillium chrysogenum</i>	ATCC 10106	Cheese	+	+	+	+
52	<i>Penicillium citreonigrum</i> ^a	Adria 1052	Environment	+	+	+	+
53	<i>Penicillium roqueforti</i> ^g	Adria M1	Dairy	-	+	-	+
54	<i>Phoma glomerata</i> ^a	Adria M4	Food	+	+	+	+
55	<i>Phytophthora infestans</i> ^a	ATCC MYA 1113	Potato Tuber	+	+	+	+
56	<i>Purpureocillium species (lilacinum)</i> ^a	ATCC 10114	Soil	+	+	+	+
57	<i>Rhizopus oryzae</i> ^a	ATCC 9363	Soy Sauce	+	+	+	+
58	<i>Rhizopus stolonifera</i> ^a	QL 14181-2A	Not Available	+	+	+	+
59	<i>Rhodotorula mucaliginosa</i>	ATCC 66034	Not Available	+	+	+	+

60	<i>Rhodotorula graminis</i> ^a	Adria 1032	Environment	+	+	+	+
61	<i>Saccharomyces cerevisiae</i>	ATCC 7754	Not Available	+	+	+	+
62	<i>Saccharomyces lactis</i>	Microbiologics	Not Available	+	+	+	+
63	<i>Scopulariopsis acremonium</i>	ATCC 58636	Chicken house soil	+	+	+	+
64	<i>Talaromyces pinophilus</i> (<i>Penicillium pinophilum</i>) ^a	NRRL 11797	Corn	+	+	+	+
65	<i>Trichoderma virens</i>	ATCC 9645	Not Available	+	+	+	+
66	<i>Trichoderma viride</i> ^a	Adria M2	Food	-	+	-	+
67	<i>Ustilago spp.</i>	Microbiologics	Not Available	+	+	+	+
68	<i>Yarrowia lipolytica</i> ^a	CLIB 183	Food	+	+	+	+
69	<i>Yarrowia lipolytica</i>	Neogen Culture collection ^d	Cheese	+	+	+	+
70	<i>Zygosaccharomyces bailii</i> (re-classified as <i>Zygosaccharomyces parabaillii</i>)	ATCC MYA-4549	Salad Dressing	-	+	-	+
71	<i>Zygosaccharomyces rouxii</i>	ATCC 28253	Processed prunes	-	+	-	+

^aStrain tested by Q Laboratories to satisfy AOAC SMPR 2021.XXX⁸ requirements. Strains without footnote “a” or “g” were tested as part of AOAC PTM 121301 and AOAC OMA 2014.05.

^bATCC = American Type Culture Collection, Manassas, VA.

^cThe “+” symbol indicates typical colony morphology observed on Petrifilm YM Plates and the “-” symbol indicates no growth observed on Petrifilm RYM Plates.

^dStrain was tested by Q Laboratories and was also part of the of AOAC PTM 121301 and AOAC OMA 2014.05.

^eStrain purchased from Microbiologics, source detail was taken from AOAC PTM 121301 (5).

^fPrefer a higher temperature for growth. These molds are visible on the Rapid Yeast and Mold plates within 48 h at 28°C but require 60 h to grow at 25°C.

^gStrain tested by ADRIA Développement for NF Validation by AFNOR certification in comparison to ISO 21527. Strains added to satisfy AOAC SMPR 2021.XXX⁸ requirements. Strains without footnote “a” or “g” were tested as part of AOAC PTM 121301 and AOAC OMA 2014.05.

^hAdria = ADRIA Développement Culture Collection, ZA Creac’h Gwen F-29196 QUIMPER Cedex.

ⁱQL = Q Laboratories Culture Collection, Cincinnati, OH.

^jNeogen = Neogen Culture Collection, St. Paul, MN, source detail was taken from AOAC PTM 121301 (5).

^k*Paecilomyces variotii* were visible on the Rapid Yeast and Mold plates at 72 h at 25°C.

Table 3. Exclusivity Results for the Petrifilm RYM Plates (3)

No.	Organism	Source	Origin	25°C 48 h Results	25°C 60 h Results	28°C 48 h Results	28°C 60 h Results
1	<i>Aeromonas hydrophila</i>	ATCC ^a 7965	Not Available	- ^c	-	-	-
2	<i>Bacillus subtilis</i>	ATCC 6633	Not Available	-	-	-	-
3	<i>Bacillus subtilis</i> ^b	Adria ^d 863	Not Available	-	-	-	-
4	<i>Bacillus weihenstephanensis</i> ^b	Adria 778	Not Available	-	-	-	-
5	<i>Brocotix thermospacta</i> ^b	EN ^d 15/29	Not Available	-	-	-	-
6	<i>Bruttiaux agrestis</i> ^b	Adria 117	Not Available	-	-	-	-
7	<i>Carnobacterium piscicola</i> ^b	Adria 369	Not Available	-	-	-	-
8	<i>Citrobacter braakii</i> ^e	ATCC 43162	Clinical Isolate	-	-	-	-
9	<i>Corynebacterium spp</i> ^b	Adria 361	Not Available	-	-	-	-
10	<i>Edwardsiella tarda</i> ^e	ATCC 15947	Human Feces	-	-	-	-
11	<i>Enterobacter cloacae</i> ^e	ATCC 13047	Spinal Fluid	-	-	-	-
12	<i>Enterococcus faecalis</i>	ATCC 14506	Not Available	-	-	-	-
13	<i>Enterococcus faecalis</i> ^b	Adria 288	Not Available	-	-	-	-
14	<i>Erwinia amylovora</i> ^e	ATCC 51852	Plant	-	-	-	-
15	<i>Escherichia coli</i>	ATCC 25922	Clinical Isolate	-	-	-	-
16	<i>Escherichia coli</i> O157:H7 ^e	ATCC 43895	Raw Hamburger	-	-	-	-
17	<i>Hafnia alvei</i> ^e	ATCC 51815	Milk	-	-	-	-
18	<i>Klebsiella oxytoca</i> ^e	ATCC 43165	Clinical Isolate	-	-	-	-
19	<i>Lactobacillus plantarum</i> ^b	Adria 70	Not Available	-	-	-	-
20	<i>Leclecia adecarboxylata</i> ^b	Adria 707	Not Available	-	-	-	-
21	<i>Leuconostoc oenos</i> ^b	Adria 73	Not Available	-	-	-	-
22	<i>Listeria innocua</i> ^b	Adria 644	Not Available	-	-	-	-
23	<i>Micrococcus leteus</i> ^b	Adria 438	Not Available	-	-	-	-
24	<i>Moraxella</i> ^b	Adria 51.11	Not Available	-	-	-	-
25	<i>Plesiomonas shigelloides</i> ^b	Adria 673	Not Available	-	-	-	-
26	<i>Pseudomonas fluorescens</i> ^b	Adria 16	Not Available	-	-	-	-
27	<i>Pseudomonas putida</i> ^b	Adria 4	Not Available	-	-	-	-
28	<i>Ralstonia pickettii</i> ^e	ATCC 27511	Clinical Isolate	-	-	-	-
29	<i>Rhanella aqatilis</i> ^b	Adria 67	Not Available	-	-	-	-
30	<i>shewanella putrefaciens</i> ^b	EN 15/34	Not Available	-	-	-	-
31	<i>Staphylococcus aureus</i> ^b	Adria 904	Not Available	-	-	-	-
32	<i>Staphylococcus epidermidis</i> ^b	Adria 931	Not Available	-	-	-	-

^aATCC = American Type Culture Collection, Manassas, VA.

^bStrain tested by ADRIA Développement for NF Validation by AFNOR certification in comparison to ISO 21527. Strains without a footnote were tested as part of AOAC PTM 121301 and AOAC OMA 2014.05.

^cThe “-” symbol indicates no growth observed on Petrifilm RYM Plates.

^dAdria = ADRIA Développement Culture Collection, ZA Creac’h Gwen F-29196 QUIMPER Cedex.

^eStrain tested by Q Laboratories to satisfy AOAC SMPR 2021.XXX⁸ requirements. Strains without a footnote were tested as part of AOAC PTM 121301 and AOAC OMA 2014.05.

Table 5. Matrix study: Petrifilm RYM Plate 25°C at 72 h vs DRBC – Difference of Means (3)

Matrix	Cont. level	Petrifilm RYM – 25°C			DRBC			DOM ^d	90% CI ^e		95%	
		Mean ^a	s _r ^b	RSD _r ^c	Mean	s _r	RSD _r		LCL ^f	UCL ^g	LCL	UCL
Cannabis Flower	Low	3.281	0.094	2.86	3.688	0.218	5.91	-0.407	-0.577	-0.236	-0.629	-0.185
	Med	3.816	0.042	1.10	3.906	0.047	1.20	-0.09	-0.12	-0.06	-0.129	-0.051
	High	5.139	0.063	1.23	5.145	0.204	3.97	-0.005	0.143	0.132	-0.184	0.174

^aMean of five replicate portions, after logarithmic transformation: Log₁₀[CFU/g + (0.1)f].^bRepeatability standard deviation.^cRSD_r = Relative standard deviation of repeatability.^dDOM = Difference of Means.^eCI = Confidence Interval for DOM.^fLCL = Lower confidence limit for DOM.^gUCL = Upper confidence limit for DOM.**Table 6. Matrix study: Petrifilm RYM Plate 28°C at 72 h vs DRBC – Difference of Means (3)**

Matrix	Cont. level	Petrifilm RYM – 28°C			DRBC			DOM ^d	90% CI ^e		95%	
		Mean ^a	s _r ^b	RSD _r ^c	Mean	s _r	RSD _r		LCL ^f	UCL ^g	LCL	UCL
Cannabis Flower	Low	3.181	0.067	2.11	3.688	0.218	5.91	-0.507	-0.682	-0.332	-0.736	-0.279
	Med	3.697	0.039	1.05	3.906	0.047	1.20	-0.209	-0.284	-0.135	-0.306	-0.112
	High	5.065	0.208	4.11	5.145	0.204	3.97	-0.08	-0.172	0.013	-0.200	0.041

^aMean of five replicate portions, after logarithmic transformation: Log₁₀[CFU/g + (0.1)f].^bRepeatability standard deviation.^cRSD_r = Relative standard deviation of repeatability.^dDOM = Difference of Means.^eCI = Confidence Interval for DOM.^fLCL = Lower confidence limit for DOM.^gUCL = Upper confidence limit for DOM.**DISCUSSION OF MODIFICATION APPROVED JANUARY 2022 (4)**

The Petrifilm RYM Plate evaluated in this study proved to be a reliable alternative at both incubation times and temperatures to the FDA/BAM Chapter 18 reference method for enumeration of yeast and mold on stainless steel, rubber, and sealed concrete. The results of the statistical analysis using the difference of means with calculated 90 and 95% confidence intervals indicated equivalence between the Petrifilm RYM Plate method and the reference method in all contamination levels analyzed.

The method allows the user to obtain accurate results within 48–60 h in the matrixes evaluated for the presence of yeast and mold when incubated at 25 ± 1°C or 28 ± 1°C. Sample preparation is paired with the reference method, as is the workflow for plating onto the Petrifilm RYM Plate. The Petrifilm Flat Spreader was easy to use and aided the spread of the inoculum across the entire gel surface of the plate. Interpretation and colony counting was straight forward just as with traditional plates. The Petrifilm RYM Plate method required no additional agar or Petri dishes, creating an easier workflow by cutting down on supplies, sample plating time, and most noticeably, occupying less space in the incubator.

Table 2. Matrix study: Petrifilm RYM Plate (25°C 48 h) vs BAM Ch 18 results (4)

Matrix	Cont. level ^a	Petrifilm RYM Plate results		FDA/BAM Ch 18 results ^d		DOM. ^e	SE ^f	90% CI ^g		95% CI	
		Mean ^b	sr ^c	Mean	sr			LCL ^h	UCL ⁱ	LCL	UCL
Stainless steel/ <i>Aspergillus</i>	Non	0	0	0	0	0	0	0	0	0	0
<i>flavus</i> ATCC ^j	Low	1.004	0.000	1.124	0.164	-0.120	0.073	-0.276	0.037	-0.323	0.084
9643	Med	2.054	0.061	2.445	0.055	-0.391	0.037	-0.461	-0.321	-0.478	-0.304
	High	3.044	0.076	3.254	0.105	-0.211	0.031	-0.278	-0.144	-0.298	-0.123
Rubber/ <i>Penicillium</i>	Non	0	0	0	0	0	0	0	0	0	0
<i>chrysogenum</i>	Low	1.745	0.066	1.745	0.066	0.000	0.021	-0.045	0.045	-0.059	0.059
ATCC 10106	Med	2.794	0.109	2.811	0.115	-0.018	0.054	-0.132	0.096	-0.166	0.131
	High	3.320	0.088	3.440	0.173	-0.121	0.057	-0.242	0.001	-0.279	0.038
Sealed concrete/ <i>Candida</i>	Non	0	0	0	0	0	0	0	0	0	0
<i>lusitanae</i> QL ^k	Low	1.752	0.256	1.722	0.250	0.029	0.145	-0.281	0.339	-0.374	0.433
15166-2	Med	2.691	0.182	2.623	0.190	0.068	0.058	-0.056	0.193	-0.094	0.230
	High	3.748	0.146	3.727	0.143	0.021	0.063	-0.114	0.156	-0.155	0.197

^aAll surfaces are artificially contaminated, 100 cm² test areas. Non indicates non-inoculated.^bMean of five replicate portions, after logarithmic transformation: Log₁₀[CFU/g + (0.1)f], where f is the reported CFU/unit corresponding to the smallest reportable result and unit is the reported unit of measure.^cRepeatability standard deviation.^dFDA/BAM Ch 18, Yeasts, Molds and Mycotoxins.^eDifference of means between the candidate and reference methods.^fStandard error.^gConfidence interval.^hLower confidence limit for difference of means.ⁱUpper confidence limit for difference of means.^jAmerican Type Culture Collection, Manassas, VA.^kQ Laboratories Culture Collection, Cincinnati, OH.**Table 3. Matrix study: Petrifilm RYM Plate (25°C 60 h) vs BAM Ch 18 results (4)**

Matrix	Cont. level ^a	Petrifilm RYM Plate results		FDA/BAM Ch 18 results ^d		DOM. ^e	SE ^f	90% CI ^g		95% CI	
		Mean ^b	sr ^c	Mean	sr			LCL ^h	UCL ⁱ	LCL	UCL
Stainless steel/ <i>Aspergillus</i>	Non	0	0	0	0	0	0	0	0	0	0
<i>flavus</i> ATCC ^j	Low	1.004	0.000	1.124	0.164	-0.120	0.073	-0.276	0.037	-0.323	0.084
9643	Med	2.086	0.030	2.445	0.055	-0.359	0.027	-0.416	-0.301	-0.434	-0.284
	High	3.134	0.078	3.254	0.105	-0.120	0.039	-0.203	-0.037	-0.228	-0.012
Rubber/ <i>Penicillium</i>	Non	0	0	0	0	0	0	0	0	0	0
<i>chrysogenum</i>	Low	1.772	0.083	1.745	0.066	0.027	0.034	-0.044	0.099	-0.066	0.121
ATCC 10106	Med	2.786	0.093	2.811	0.115	-0.026	0.056	-0.146	0.094	-0.182	0.130
	High	3.405	0.061	3.440	0.173	-0.035	0.056	-0.155	0.085	-0.191	0.121
Sealed concrete/ <i>Candida</i>	Non	0	0	0	0	0	0	0	0	0	0
<i>lusitanae</i> QL ^k	Low	1.761	0.265	1.722	0.250	0.038	0.149	-0.280	0.356	-0.376	0.452
15166-2	Med	2.690	0.179	2.623	0.190	0.068	0.059	-0.058	0.193	-0.096	0.231
	High	3.742	0.140	3.727	0.143	0.015	0.059	-0.111	0.141	-0.150	0.179

^aAll surfaces are artificially contaminated, 100 cm² test areas. Non indicates non-inoculated.^bMean of five replicate portions, after logarithmic transformation: Log₁₀[CFU/g + (0.1)f], where f is the reported CFU/unit corresponding to the smallest reportable result and unit is the reported unit of measure.^cRepeatability standard deviation.^dFDA/BAM Ch 18, Yeasts, Molds and Mycotoxins.^eDifference of means between the candidate and reference methods.^fStandard error.^gConfidence interval.^hLower confidence limit for difference of means.ⁱUpper confidence limit for difference of means.^jAmerican Type Culture Collection, Manassas, VA.^kQ Laboratories Culture Collection, Cincinnati, OH.

Table 4. Matrix study: Petrifilm RYM Plate (28°C 48 h) vs BAM Ch 18 results (4)

Matrix	Cont. level ^a	Petrifilm RYM Plate results		FDA/BAM Ch 18 results ^d		DOM. ^e	SE ^f	90% CI ^g		95% CI	
		Mean ^b	S _r ^c	Mean	S _r			LCL ^h	UCL ⁱ	LCL	UCL
Stainless steel/ <i>Aspergillus flavus</i> ATCC ^j 9643	Non	0	0	0	0	0	0	0	0	0	0
	Low	1.004	0.000	1.124	0.164	-0.120	0.073	-0.276	0.037	-0.323	0.084
	Med	2.105	0.050	2.445	0.055	-0.339	0.034	-0.411	-0.268	-0.433	-0.246
	High	3.109	0.111	3.254	0.105	-0.145	0.037	-0.225	-0.066	-0.249	-0.042
Rubber/ <i>Penicillium chrysogenum</i> ATCC 10106	Non	0	0	0	0	0	0	0	0	0	0
	Low	1.761	0.062	1.745	0.066	0.016	0.035	-0.059	0.090	-0.081	0.113
	Med	2.781	0.100	2.811	0.115	-0.030	0.047	-0.129	0.069	-0.159	0.099
	High	3.312	0.101	3.440	0.173	-0.128	0.070	-0.278	0.022	-0.323	0.067
Sealed concrete/ <i>Candida lusitanae</i> QL ^k 15166-2	Non	0	0	0	0	0	0	0	0	0	0
	Low	1.788	0.232	1.722	0.250	0.066	0.055	-0.053	0.184	-0.088	0.220
	Med	2.723	0.144	2.623	0.190	0.100	0.055	-0.017	0.217	-0.053	0.252
	High	3.627	0.301	3.727	0.143	-0.100	0.097	-0.306	0.106	-0.368	0.168

^aAll surfaces are artificially contaminated, 100 cm² test areas. Non indicates non-inoculated.^bMean of five replicate portions, after logarithmic transformation: Log₁₀[CFU/g + (0.1)f], where f is the reported CFU/unit corresponding to the smallest reportable result and unit is the reported unit of measure.^cRepeatability standard deviation.^dFDA/BAM Ch 18, Yeasts, Molds and Mycotoxins.^eDifference of means between the candidate and reference methods.^fStandard error.^gConfidence interval.^hLower confidence limit for difference of means.ⁱUpper confidence limit for difference of means.^jAmerican Type Culture Collection, Manassas, VA.^kQ Laboratories Culture Collection, Cincinnati, OH.**Table 5. Matrix study: Petrifilm RYM Plate (28°C 60 h) vs BAM Ch 18 results (4)**

Matrix	Cont. level ^a	Petrifilm RYM Plate results		FDA/BAM Ch 18 results ^d		DOM. ^e	SE ^f	90% CI ^g		95% CI	
		Mean ^b	S _r ^c	Mean	S _r			LCL ^h	UCL ⁱ	LCL	UCL
Stainless steel/ <i>Aspergillus flavus</i> ATCC ^j 9643	Non	0	0	0	0	0	0	0	0	0	0
	Low	1.004	0.000	1.124	0.164	-0.120	0.073	-0.276	0.037	-0.323	0.084
	Med	2.164	0.085	2.445	0.055	-0.281	0.042	-0.370	-0.192	-0.397	-0.165
	High	3.154	0.103	3.254	0.105	-0.100	0.030	-0.164	-0.036	-0.183	-0.017
Rubber/ <i>Penicillium chrysogenum</i> ATCC 10106	Non	0	0	0	0	0	0	0	0	0	0
	Low	1.766	0.119	1.745	0.066	0.021	0.049	-0.083	0.126	-0.115	0.158
	Med	2.772	0.105	2.811	0.115	-0.040	0.045	-0.136	0.057	-0.166	0.086
	High	3.341	0.119	3.440	0.173	-0.099	0.074	-0.256	0.058	-0.303	0.105
Sealed concrete/ <i>Candida lusitanae</i> QL ^k 15166-2	Non	0	0	0	0	0	0	0	0	0	0
	Low	1.788	0.232	1.722	0.250	0.066	0.055	-0.053	0.184	-0.088	0.220
	Med	2.735	0.149	2.623	0.190	0.112	0.058	-0.011	0.236	-0.049	0.273
	High	3.723	0.168	3.727	0.143	-0.005	0.039	-0.087	0.078	-0.111	0.102

^aAll surfaces are artificially contaminated, 100 cm² test areas. Non indicates non-inoculated.^bMean of five replicate portions, after logarithmic transformation: Log₁₀[CFU/g + (0.1)f], where f is the reported CFU/unit corresponding to the smallest reportable result and unit is the reported unit of measure.^cRepeatability standard deviation.^dFDA/BAM Ch 18, Yeasts, Molds and Mycotoxins.^eDifference of means between the candidate and reference methods.^fStandard error.^gConfidence interval.^hLower confidence limit for difference of means.ⁱUpper confidence limit for difference of means.^jAmerican Type Culture Collection, Manassas, VA.^kQ Laboratories Culture Collection, Cincinnati, OH.

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