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CHAPTER

17.2.11 AOAC Official Method℠ 2014.05 Enumeration of Yeast and Mold in Foods, Select Surfaces, and Dried Cannabis Flower: Neogen® Petrifilm® Rapid Yeast and Mold (RYM) Count Plate

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EP 菌粒

First Action 2014

Final Action 2017

Revised First Action 2022 (for Cannabis Flower, THC > 0.3%, and Select Surfaces Only)

[Applicable to enumeration of yeast and mold in the following high-water activity matrixes: yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, raw frozen ground beef patties (77% lean), ready-to-eat deli sandwiches, and sliced apples; low-water activity matrixes: raw almonds, dehydrated soup, and dried cannabis flower (THC > 0.3%); and environmental surfaces: stainless steel, sealed concrete, and rubber.]

Caution: After use, diluents and Petrifilm Rapid Yeast and Mold (RYM) Count Plates may contain microorganisms that may be potential biohazard as several foodborne molds can produce toxic metabolites known as mycotoxins. If further identification of mold species is required, appropriate personal protective equipment (PPE) should be used when top film is retracted and exposure to spores or mycotoxins may occur. When testing is complete, follow current industry standards for disposal of contaminated waste. Consult material safety data sheet for additional information and local regulations for disposal. For information on potential biohazards, refer to *Biosafety in Microbiological and Biomedical Laboratories*, 6th Ed., Section VIII-B: Fungal Agents.

Petrifilm RYM Plates contain chloramphenicol and chlortetracycline, potent broad spectrum antibiotic drugs commonly used in yeast and mold enumeration. The drugs, when used in humans, are associated with many toxic effects. Care should be taken to avoid coming into direct contact with the gel on the plates.

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See Tables 2014.05A and B for a summary of results of the interlaboratory study supporting acceptance of the method. Results for each collaborating laboratory's aerobic plate count analysis for each matrix are shown in Table 2014.05C.

See Table 2014.05D for results of matrix studies for cannabis flower supporting extension of the method.

See Table 2014.05E for results of matrix studies for selected environmental surfaces supporting extension of the method.

See additional tables in the *Journal of AOAC INTERNATIONAL* for detailed results of the interlaboratory study [*J. AOAC Int.* **98**, 767(2015) DOI: 10.5740/jaoacint.15-006].

A Principle

The Petrifilm RYM Plate is a sample-ready culture medium system, which contains nutrients supplemented with antibiotics, cold-water-soluble gelling agent, and indicator system that facilitates yeast and mold enumeration. Petrifilm RYM Plate are used for enumeration of yeast and mold in as little as 48 h in the food and beverage industries and 60 to 72 h in the cannabis industry. Neogen Corp. is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

B Apparatus and Reagents

(a) *Petrifilm Rapid Yeast and Mold Count Plate.*—Cat. Nos. 6475/6477; Product SKUs 700002138/700002148 (Neogen Corp., Lansing, MI, USA, www.neogen.com).

(b) *Sterile diluents.*—0.1% Peptone water (PW) or Butterfield's phosphate buffer diluent (BPBD).

(c) *Pipets.*—Capable of 1000 µL or serological pipet.

(d) *Sterile pipet tips.*—Capable of 1000 µL.

(e) *Stomacher.*—Seward or equivalent.

(f) *Filter stomacher bags.*—Seward or equivalent.

(g) *Petrifilm Flat Spreader.*—Cat. No. 6425; Product SKU 700002099 (Neogen Corp.).

(h) *Swab Sampler with 10 mL Letheen broth.*—Cat. No. RS96010LET; Product SKU 700002039 (Neogen Corp.) or equivalent.

(i) *Incubators.*—Capable of maintaining 25 ± 1°C and 28 ± 1°C and having solid front to maintain dark interior.

(j) *Refrigerator.*—Capable of maintaining 2–8°C, for storing the Petrifilm RYM Plates.

(k) *Standard colony counter or illuminated magnifier.*

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C General Instructions

(a) Store unopened Petrifilm RYM Plate pouches refrigerated or frozen (−20 to 8°C/−4 to 46°F). Just prior to use, allow unopened pouches to come to room temperature before opening (20–25°C/<60% relative humidity; RH). Return unused Petrifilm RYM Plates to pouch. Seal by folding end of pouch over and applying adhesive tape. To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches in cool dry place (20–25°C/<60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of Petrifilm RYM Plates be stored in freezer if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where RH exceeds 60% (with the exception of air-conditioned premises).
To store opened pouches in freezer, place Petrifilm RYM Plates in sealable container.
Post-incubation Petrifilm RYM Plates can be stored at −10 to −20°C for up to 7 days.

(b) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

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D Sample Preparation

(a) Aseptically prepare 1:10 dilution of each test portion.

(1) *Dairy products.*—Pipet 11 mL or weigh 11 g sample into 99 mL sterile 0.1% PW. Shake 25 times to homogenize.

(2) *All other foods.*—Weigh out 25 g sample from test portion into sterile stomacher bag and dilute with 225 mL 0.1% PW; stomach at high speed to homogenize.

(3) *Dried cannabis flower (THC >0.3%).*—Weigh out 10 g sample from test portion into sterile stomacher bag and dilute with 90 mL sterile 0.1% PW. Shake 25 times to homogenize.

(4) *Environmental surfaces.*—Mix or shake swab in Letheen broth vigorously.

(b) Prepare 10-fold serial dilutions in 0.1% PW or BPBD. Environmental surface samples may be plated directly as needed.

(c) Place Petrifilm RYM Plate on flat, level surface for each dilution to be tested.

(d) Lift top of film. Dispense 1 mL of each dilution onto center of bottom film of each plate.

(e) Roll film down onto sample.

(f) Place Petrifilm Flat Spreader on center of plate. Press gently on center of spreader to distribute sample evenly. Spread inoculum over entire Petrifilm RYM Plate growth area before gel is formed. *Do not slide spreader across film.*

(g) Remove spreader and leave plate undisturbed for at least 1 min to permit gel to form.

(h) Incubate Petrifilm RYM Plates at 25 or 28°C in horizontal position with clear side up in stacks of no more than 40.

(1) *For food or environmental samples.*—Enumerate plates after 48 h of incubation. If colonies appear faint, allow for additional 12 h of incubation time for enhanced interpretation. If 60 h time point for interpretation is not convenient, extending incubation time to 72 h is acceptable alternative.

(2) *For dried cannabis flower.*—Enumerate plates at 60 to 72 h of incubation.

(i) Petrifilm RYM Plates can be counted using standard colony counter with the use of backlight or illuminated magnifier to assist with estimated enumeration. Do not count colonies on foam dam since they are removed from the nutrient medium.

(j) Yeast colonies appear raised and small with defined edges. Colonies may appear pink/tan or blue/green in color.

(k) Mold colonies appear flat with dark center and diffused edges. Colonies may appear blue/green to variable upon prolonged incubation. *See* Table 2014.05F for yeast and mold appearance.

(l) The circular growth area is approximately 30 cm^2. Plates containing >150 colonies can be either estimated or recorded as too numerous to count (TNTC). Estimation can only be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When

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the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be at user discretion.

(m) Samples may occasionally show interference on the Petrifilm RYM Plates, for example:

(1) Uniform blue background color (often seen from organisms used in cultured products). These should not be counted as TNTC.

(2) Intense pinpoint blue specks (often seen with spices, granulated products, or dried cannabis flower).

(n) Report final results as colony-forming units/gram (CFU/g).

(o) If required, colonies may be isolated for further identification by direct microscopy or biochemical analysis. Lift top film and pick colony from gel.

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Table 2014.05A. Interlaboratory study results of Neogen Petrifilm RYM versus FDA-BAM and ISO 21527 methods for frozen raw ground beef patties

	Contam. level	Neogen Petrifilm RYM method				FDA-BAM/ISO 21527 methods[a]				*P*-value[d]	Difference of means	Reverse transformed mean difference[e]
		N[b]	Mean[c]	s_r	s_R	*N*	Mean	s_r	s_R			
25°C, 48 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.12	0.41	0.41	11(1)	2.07	0.36	0.38	0.5323	0.05	14.34
	Medium	11(0)	3.52	0.10	0.10	11(0)	3.47	0.09	0.11	0.1637	0.05	360.10
	High	11(0)	4.65	0.13	0.14	11(0)	4.59	0.10	0.14	0.2266	0.06	5763.84
25°C, 60 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.14	0.36[f]	0.37	11(1)	2.07	0.36	0.38	0.3773	0.07	20.55
	Medium	11(0)	3.52	0.10	0.10	11(0)	3.47	0.09	0.11	0.1573	0.05	360.10
	High	11(0)	4.65	0.14	0.15	11(0)	4.59	0.10	0.14	0.1750	0.06	5763.84
28°C, 48 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.17	0.29[f]	0.30	11(1)	2.07	0.36	0.38	0.1391	0.10	30.42
	Medium	11(0)	3.53	0.10	0.10	11(0)	3.47	0.09	0.11	0.0824	0.06	437.23
	High	11(0)	4.67	0.08[f]	0.11	11(0)	4.59	0.10	0.14	0.0966	0.08	7869.00

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28°C, 60 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.16	0.29[f]	0.29	11(1)	2.07	0.36	0.38	0.1843	0.09	27.05
	Medium	11(0)	3.53	0.09	0.10	11(0)	3.47	0.09	0.11	0.1095	0.06	437.23
	High	11(0)	4.67	0.08[f]	0.11	11(0)	4.59	0.10	0.14	0.1088	0.08	7869.00

a Samples were analyzed by harmonized FDA-BAM Chapter 18 and ISO 21527 methods using 0.1% peptone as the sample diluent.

b *N* = Number of laboratories that reported complete results. Outliers are in parentheses.

c Log_{10} yeast and mold CFU/g.

d Significant difference (P <0.05).

e Results presented as CFU/g.

f Results indicate that the candidate method is more repeatable than the reference methods. s_r = Repeatability standard deviation; s_R = reproducibility standard deviation.

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Table 2014.05B. Interlaboratory study results of Neogen Petrifilm RYM versus FDA-BAM and ISO 21527 methods for raw almonds

	Contam. level	Neogen Petrifilm RYM method				FDA-BAM/ISO 21527 methods[a]				*P*-value[d]	Difference of means	Reverse transformed mean difference[e]
		N[b]	Mean[c]	s_r	s_R	*N*	Mean	s_r	s_R			
25°C, 48 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.45	0.17[f]	0.26	14(0)	1.55	0.19	0.34	0.4165	0.10	−7.30
	Medium	14(1)	2.12	0.26	0.39	14(0)	2.21	0.20	0.24	0.3322	0.09	−30.36
	High	14(2)	3.00	0.18	0.49	14(1)	3.08	0.12	0.31	0.2833	0.08	−202.26
25°C, 60 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.53	0.23	0.28	14(0)	1.55	0.19	0.34	0.8391	0.02	−1.60
	Medium	14(0)	2.20	0.21	0.27	14(0)	2.21	0.20	0.24	0.7789	0.01	−3.69
	High	14(2)	3.04	0.18	0.41	14(1)	3.08	0.12	0.31	0.5418	0.04	−105.79
28°C, 48 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.58	0.16[f]	0.21	14(0)	1.55	0.19	0.34	0.7381	0.03	2.54
	Medium	14(0)	2.17	0.17[f]	0.29	14(0)	2.21	0.20	0.24	0.6139	0.04	−11.73
	High	14(2)	3.01	0.17	0.45	14(1)	3.08	0.12	0.31	0.3904	0.07	−178.97

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28°C, 60 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.60	0.17[f]	0.20	14(0)	1.55	0.19	0.34	0.5474	0.05	4.33
	Medium	14(0)	2.21	0.17[f]	0.23	14(0)	2.21	0.20	0.24	0.9483	0.00	0.00
	High	14(2)	3.03	0.18	0.42	14(1)	3.08	0.12	0.31	0.4687	0.05	−130.75

a Samples were analyzed by harmonized FDA-BAM Chapter 18 and ISO 21527 methods using 0.1% peptone as the sample diluent.

b *N* = Number of laboratories that reported complete results. Outliers are in parentheses.

c Log_{10} yeast and mold CFU/g.

d Significant difference (P <0.05).

e Results presented as CFU/g.

f Results indicate that the candidate method is more repeatable than the reference methods. s_r = Repeatability standard deviation; s_R = reproducibility standard deviation.

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Table 2014.05C. Results of aerobic plate count for collaborating laboratories

Lab	Frozen raw ground beef, CFU/g	Raw almonds, CFU/g
1	3.8×10^2	6.0×10^1
2	1.1×10^3	6.0×10^2
3	<10	3.0×10^1
4	Not reported	Not reported
5	2.8×10^3	2.8×10^1
6	8.0×10^1	2.2×10^1
7	9.1×10^2	1.6×10^2
8	Not reported	Not reported
9	9.0×10^2	2.0×10^2
10	1.3×10^3	4.0×10^2
11	>2500	1.0×10^1
12	Not reported	7.0×10^1
13	9.5×10^1	1.0×10^1
14	7.3×10^2	2.3×10^2
15	3.7×10^2	8.0×10^1

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Table 2014.05D. Matrix studies: Neogen Petrifilm RYM Plate vs Dichloran Rose Bengal Chloramphenicol (DRBC) agar–difference of means

Matrix	Contamination level	Petrifilm RYM		DRBC		DOM[c]	SE[d]	90% CI[e]		95%	
		Mean[a]	s_r [b]	Mean	s_r			LCL[f]	UCL[g]	LCL	UCL
Petrifilm RYM Plate 25°C at 72 h											
Cannabis flower[h]	Low	3.281	0.094	3.688	0.218	−0.407	0.106	−0.577	−0.236	−0.629	−0.185
	Med	3.816	0.042	3.906	0.047	−0.09	0.028	−0.12	−0.06	−0.129	−0.051
	High	5.139	0.063	5.145	0.204	−0.005	0.064	0.143	0.132	−0.184	0.174
Cannabis flower[i]	Low	2.850	0.222	3.151	0.211	−0.301	0.121	−0.468	0.047	−0.403	−0.199
	High	5.435	0.180	5.539	0.143	−0.105	0.075	−0.264	0.055	−0.379	−0.222
Petrifilm RYM Plate 25°C at 60 h											
Cannabis flower[h]	Low	2.788	0.217	3.151	0.211	−0.363	0.113	−0.427	−0.299	−0.446	−0.280
	High	5.392	0.166	5.539	0.143	−0.147	0.072	−0.302	0.007	−0.348	0.054
Petrifilm RYM Plate 28°C at 72 h											
Cannabis flower[h]	Low	3.181	0.067	3.688	0.218	−0.507	0.082	−0.682	−0.332	−0.736	−0.279
	Med	3.697	0.039	3.906	0.047	−0.209	0.035	−0.284	−0.135	−0.306	−0.112
	High	5.065	0.208	5.145	0.204	−0.08	0.130	−0.172	0.013	−0.200	0.041
Cannabis flower[i]	Low	2.838	0.214	3.061	0.226	−0.222	0.127	−0.493	0.048	−0.575	0.130

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	High	5.450	0.176	5.539	0.143	−0.089	0.049	−0.194	0.016	−0.225	0.047
Petrifilm RYM Plate 28°C at 60 h											
Cannabis flower[h]	Low	2.817	0.204	3.061	0.226	−0.244	0.114	−0.486	−0.001	−0.560	0.072
	High	5.432	0.188	5.539	0.143	−0.107	0.054	−0.221	0.007	−0.256	0.042

a Mean of five replicate portions, after logarithmic transformation: $Log_{10}[CFU/g + (0.1)f]$.

b s_r = Repeatability standard deviation.

c DOM = Difference of means.

d SE = Standard error on the mean difference for paired analysis.

e CI = Confidence interval for DOM.

f LCL = Lower confidence limit for DOM.

g UCL = Upper confidence limit for DOM.

h Samples tested at North Coast Laboratories.

i Samples tested at Aurum Labs.

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Table 2014.05E. Matrix study: Neogen Petrifilm RYM Plate vs BAM Ch. 18 results

Matrix	Contam. level[a]	Petrifilm RYM		FDA/BAM Ch. 18[d]		DOM[e]	SE[f]	90% CI[g]		95% CI	
		Mean[b]	s_r[c]	Mean	s_r			LCL[h]	UCL[i]	LCL	UCL
3M Petrifilm RYM Plate (25°C, 48 h)											
Stainless steel/*Aspergillus flavus* ATCC[j] 9643	Low	1.004	0.000	1.124	0.164	−0.120	0.073	−0.276	0.037	−0.323	0.084
	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/*Penicillium chrysogenum* ATCC 10106	Low	1.745	0.066	1.745	0.066	0.000	0.021	−0.045	0.045	−0.059	0.059
	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/*Candida lusitaniae* QL[k] 15166-2	Low	1.752	0.256	1.722	0.250	0.029	0.145	−0.281	0.339	−0.374	0.433
	Med	2.691	0.182	2.623	0.190	0.068	0.058	−0.056	0.193	−0.094	0.230

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	High	3.748	0.146	3.727	0.143	0.021	0.063	–0.114	0.156	–0.155	0.197
3M Petrifilm RYM Plate (25°C, 60 h)											
Stainless steel/*Aspergillus flavus* ATCC 9643	Low	1.004	0.000	1.124	0.164	–0.120	0.073	–0.276	0.037	–0.323	0.084
	Med	2.086	0.030	2.445	0.055	–0.359	0.027	–0.416	–0.301	–0.434	–0.284
	High	3134	0.078	3.254	0.105	–0.120	0.039	–0.203	–0.037	–0.228	–0.012
Rubber/*Penicillium chrysogenum* ATCC 10106	Low	1.772	0.083	1.745	0.066	0.027	0.034	–0.044	0.099	–0.066	0.121
	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/*Candida lusitaniae* QL 15166-2	Low	1.761	0.265	1.722	0.250	0.038	0.149	–0.280	0.356	–0.376	0.452
	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
3M Petrifilm RYM Plate (28°C, 48 h)											

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Stainless steel/*Aspergillus flavus* ATCC 9643	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/*Penicillium chrysogenum* ATCC 10106	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/*Candida lusitaniae* QL 15166-2	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
3M Petrifilm RYM Plate (28°C, 60 h)											
Stainless steel/*Aspergillus flavus* ATCC 9643	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

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	High	3.154	0.103	3.254	0.105	–0.100	0.030	–0.164	–0.036	–0.183	–0.017
Rubber/*Penicillium chrysogenum* ATCC 10106	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/*Candida lusitaniae* QL 15166-2	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	2.723	0.168	2.727	0.143		0.039		0.078		0.102

a All surfaces are artificially contaminated, 100 cm^2 test areas.

b Mean of five replicate portions, after logarithmic transformation: $Log_{10}[CFU/g + (0.1)f]$ where f is the smallest reportable result.

c s_r = Repeatability standard deviation.

d FDA/BAM Ch. 18, Yeasts, Molds, and Mycotoxins.

e DOM = Difference of means between candidate and reference methods.

f SE = Standard error.

g CI = Confidence interval.

h LCL = Lower confidence limit for DOM.

i UCL = Upper confidence limit for DOM.

j ATCC = American Type Culture Collection, Manassas, VA, USA.

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k QL = Q Laboratories Culture Collection, Cincinnati, OH, USA.

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Table 2014.05F. Appearance of yeast and mold on Neogen Petrifilm RYM Plates

Yeast colonies	Mold colonies
Small	Large
Defined edges	Diffused edges
Pink/tan to blue/green in color	Blue/green to variable upon prolonged incubation
Appear raised (3-dimensional)	Appear flat
Uniform color	Dark center with diffused edges

References:

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AOAC SMPR 2021.009 (Yeast and Mold Count Enumeration in Cannabis and Cannabis Products) https://www.aoac.org/wp-content/uploads/2021/06/SMPR-2021_009.pdf

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