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CHAPTER

17.2.09 AOAC Official Method SM 997.02 Yeast and Mold Counts in Foods and Dried Cannabis Flower: Dry Rehydratable Film Method Neogen[®] Petrifilm[®] Yeast and Mold Count Plate

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First Action 1997

Final Action 2000

Revised First Action 2021 (for Cannabis Flower, THC >0.3%, Only)

[Applicable to enumeration of total yeasts and molds in foods and dried cannabis flower (THC >0.3%).]

See Tables 997.02A and B for results of the interlaboratory study supporting acceptance of the method.

Table 997.02A Interlaboratory study results for determination of mold count in foods by dry rehydratable film method

Product	Mold level	Method	Mean log ₁₀ colony count	s _r	s _R	RSD _r , %	RSD _R , %	r ^a	R ^b
Orange juice	Low	PYM ^c	2.50	0.13	0.17	5.05	6.93	0.36	0.49
		BAM ^d	2.50	0.33	0.38	13.23	15.17	0.94	1.07
	High	PYM	3.23	0.18	0.37	5.68	11.51	0.52	1.05
		BAM	3.21	0.12	0.36	3.66	11.10	0.33	1.01
Hot dog	Low	PYM	2.35	0.32	0.80	13.67	34.00	0.91	2.26
		BAM	2.20	0.08	0.98	3.44	44.69	0.21	2.78
	High	PYM	3.09	0.11	0.97	3.58	31.54	0.31	2.76
		BAM	3.06	0.19	0.98	6.18	31.89	0.54	2.76
Yogurt	Low	PYM	2.34	0.16	0.75	6.90	31.81	0.46	2.11
		BAM	2.15	0.11	0.92	5.18	42.68	0.31	2.59
	High	PYM	3.21	0.43	0.50	13.45	15.70	1.22	1.42
		BAM	3.00	0.17	0.92	5.50	30.49	0.47	2.59
Ketchup	Low	PYM	2.17	2.52	2.61	116.00	120.10	7.13	7.38
		BAM	1.90	0.27	0.67	13.93	35.08	0.75	1.88
	High	PYM	2.76	0.42	0.50	15.35	18.01	1.20	1.41
		BAM	2.78	0.18	0.80	6.32	30.50	0.50	2.40
Corn meal	Low	PYM	2.28	0.69	0.76	30.18	33.53	1.95	2.16
		BAM	2.29	0.39	0.63	17.13	27.56	1.11	1.78
	High	PYM	2.50	0.61	0.76	24.54	29.81	1.73	2.11
		BAM	2.54	0.51	0.64	20.01	25.10	1.44	1.80
Cake mix	Low	PYM	1.73	0.30	0.68	17.29	39.06	0.85	1.92
		BAM	1.57	0.52	0.82	33.35	52.29	1.48	2.31
	High	PYM	1.73	0.49	0.78	28.13	44.96	1.37	2.20
		BAM	1.71	0.31	0.77	18.26	45.01	0.88	2.18

a $r = 2.8 \times s_r$.

b $R = 2.8 \times s_R$.

c PYM = Petrifilm Yeast and Mold Count Plate.

d BAM = Method described in U.S. Food and Drug Administration's *Bacteriological Analytical Manual*, 7th Ed., AOAC INTERNATIONAL, Rockville, MD, USA.

Table 997.02B Interlaboratory study results for determination of yeast count in foods by dry rehydratable film method

Product	Yeast level	Method	Mean log ₁₀ colony count	s _r	s _R	RSD _r , %	RSD _R , %	r ^a	R ^b
Orange juice	Low	PYM ^c	1.72	0.48	0.77	28.05	44.98	1.36	2.18
		BAM ^d	1.72	0.51	0.82	29.41	47.81	1.43	2.33
	High	PYM	2.93	0.26	0.38	8.98	13.07	0.74	1.08
		BAM	2.95	0.15	0.36	5.20	12.32	0.43	1.03
Corn meal	Low	PYM	1.32	0.98	1.48	73.99	112.00	2.76	4.18
		BAM	1.49	0.81	1.45	54.00	96.88	2.28	4.09
	High	PYM	1.99	1.16	1.51	58.33	75.70	3.28	4.26
		BAM	2.27	1.08	1.32	47.85	58.15	3.07	3.73
Cake mix	Low	PYM	1.51	0.58	1.11	38.47	73.61	1.64	3.14
		BAM	1.23	0.75	1.18	61.10	96.59	2.12	3.35
	High	PYM	2.09	0.25	1.06	11.79	50.78	0.70	3.00
		BAM	2.07	0.40	1.14	19.25	55.01	1.13	3.22

a $r = 2.8 \times s_r$.

b $R = 2.8 \times s_R$.

c PYM = Petrifilm Yeast and Mold Count Plate.

d BAM = Method described in FDA *Bacteriological Analytical Manual*, 7th Ed., AOAC INTERNATIONAL, Rockville, MD, USA.



A Principle

The method uses culture plates of dry medium supplemented with antibiotics, dye to enhance visualization of growth, and cold-water-soluble gelling agent. Undiluted or diluted suspensions are added to plates at a rate of 1 mL/plate. Suspension is spread over a 30 cm² growth area. Gelling agent is allowed to solidify, plates are incubated, and yeasts and molds are counted.

B Apparatus and Reagent

- (a) *Yeast and mold (YM) count plates.*—Contain nutrients supplemented with chlortetracycline, chloramphenicol, cold-water-soluble gelling agent, and dye sensitive to presence of phosphatase (5-bromo-4-chloro-3-indolyl phosphate) that enhances visualization of yeast and mold growth. The circular growth area of a single plate contains thirty 1 × 1 cm squares outlined on a film base [available as Petrifilm Yeast and Mold Count Plates, Cat. No. 6407/6417, Product SKU 700002121/700002128, Neogen Corp. (Lansing, MI, USA, www.neogen.com)].
- (b) *Plastic spreader.*—Provided with Petrifilm plates, designed to spread suspension evenly over plate growth area.
- (c) *Pipets.*—Serological pipet or pipetting syringe accurately delivering 1.0 mL.
- (d) *Colony counter.*—Standard apparatus, Quebec model preferred, or one providing equivalent magnification (1.5×) and visibility.
- (e) *Blender.*—High-speed mechanical blender rotating at 10000–12000 rpm or stomacher.
- (f) *Sterile diluents.*—Butterfield's phosphate-buffered dilution water or 0.1% peptone water (PW).

C General Instructions

Store unopened PYM Plate pouches refrigerated or frozen at temperatures $\leq 8^{\circ}\text{C}$ (46°F). Just prior to use, allow unopened pouches to come to room temperature before opening. Return unused PYM Plates to pouch. Seal by folding the end of the pouch over and applying adhesive tape. To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches in a cool, dry place for no longer than 4 weeks. It is recommended that resealed pouches of PYM Plates be stored in a freezer (*see* product instructions) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 50% (with the exception of air-conditioned premises).

After use, plates contain viable yeast and/or mold cultures. Follow current industry standards and local regulations for disposal of biohazardous waste.

D Preparation of Test Suspension

Aseptically prepare 1:10 or greater dilution of food samples with sterile diluent. Blend or stomach 2 min and plate. Prepare additional dilutions as required. For 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. Prepare additional dilutions as required.

E Analysis

Place Petrifilm YM Count Plate on flat surface. Lift top film, hold pipet perpendicular to plate, and carefully inoculate 1 mL test suspension onto center of film base. Place top film down onto inoculum.

Lift plastic spreader using circular handle. Align center of spreader with approximate center of plate. Distribute suspension evenly using gentle downward pressure on center of spreader. *Do not slide spreader*

across film. Remove spreader and leave plate undisturbed for 1 min to let gel solidify.

Place plates in incubator in horizontal position, clear side up, in stacks not exceeding 20 units. Incubate plates 5 days at 20–25°C.

Count plates promptly after incubation period. Yeast colonies appear as blue–green or off–white in color and form small, defined colonies. Mold colonies are usually blue but may also assume their natural pigmentation (e.g., black, yellow, green). They tend to be larger and more diffuse than yeast colonies.

To calculate yeast and mold count, multiply total number of yeast and mold colonies/plate (or average number of colonies/plate if counting duplicate plates of same dilution) by the appropriate dilution factor. When counting colonies on duplicate plates of consecutive dilutions, calculate the mean number of colonies for each dilution before determining average yeast and mold count.

Estimated counts can be made on plates with >150 colonies and should be reported as estimated counts. In making such counts, determine average count/1 cm² and multiply by 30 (circular growth area is 30 cm²).

High numbers of yeast colonies may cause entire growth area to turn blue. High numbers of mold colonies may cause growth area to turn blue, black, yellow, etc. When this occurs, do not make estimated counts, but further dilute and plate test suspension to obtain a more accurate count.

References:

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