

AOAC® Official Method 2014.05

Enumeration of Yeast and Mold in Food

3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

First Action 2014

[Applicable to the enumeration of yeast and mold in the following high-water activity matrices: yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, raw frozen ground beef patties (77% lean), ready-to-eat deli sandwiches, sliced apples, and the following low-water activity matrices: raw almonds and dehydrated soup.]

See Tables **2014.05A** and **2014.05B** for a summary of results of the collaborative study. The result for each collaborating laboratory's aerobic plate count analysis for each matrix is shown in Table **2014.05C**.

See Tables 2–9 in the *J. AOAC Int.* paper for detailed results of the collaborative study.

A. Principle

The 3M™ 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. 3M Petrifilm RYM Count Plates are used for the enumeration of yeast and mold in as little as 48 hours in the food and beverage industries. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

B. Apparatus and Reagents

- **3M Petrifilm RYM Count Plate:** 25 plates/pouch; two pouches/box (3M Food Safety, St. Paul, MN, USA)
- **Sterile Diluents:** 0.1% peptone water
- **Pipets:** Capable of 1000µL or a serological pipet
- **Sterile Pipet Tips:** Capable of 1000µL
- **Stomacher:** Seward or equivalent
- **Filter Stomacher Bags:** Seward or equivalent
- **3M™ Petrifilm™ Flat Spreader**
- **Incubators:** Capable of maintaining $25 \pm 1^\circ\text{C}$ and $28 \pm 1^\circ\text{C}$ and having a solid front to maintain a dark interior
- **Refrigerator:** Capable of maintaining $2\text{--}8^\circ\text{C}$, for storing the 3M Petrifilm RYM Plates
- **L-shaped Spreaders**
- **Standard Colony Counter or Illuminated Magnifier**

C. General Instructions

- 1) Store unopened 3M Petrifilm RYM Count 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 ($20\text{--}25^\circ\text{C}$ / $<60\%$ RH). Return unused 3M Petrifilm RYM Count 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 ($20\text{--}25^\circ\text{C}$ / $<60\%$ RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M Petrifilm RYM Count Plates be stored in a freezer if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 60% (with the exception of air-conditioned premises).
- 2) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

D. Safety Precautions

After use, the diluents and The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plates may contain microorganisms that may be a potential biohazard as several foodborne molds have the ability to produce toxic metabolites known as mycotoxins. If further identification of a 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 the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal. For information on potential biohazards, reference *Biosafety in Microbiological and Biomedical Laboratories*, 5th Ed., Section VIII-B: Fungal Agents.

The 3M Petrifilm RYM Count Plates contain chloramphenicol and chlortetracycline, potent broad spectrum antibiotic drugs commonly used in yeast and mold enumeration. The drug, when used in humans, is associated with many toxic effects. Care should be taken to avoid coming into direct contact with the gel on the plates.

E. Sample Preparation

- 1) Aseptically prepare a 1:10 dilution of each test portion.
 - **Dairy Products:** Pipet 11mL or weigh 11g of sample into 99mL sterile 0.1% peptone water. Shake 25 times to homogenize.
 - **All Other Foods:** Weigh out 25g of sample from test portion into a sterile stomacher bag and dilute with 225mL of 0.1 % peptone water; stomach at high speed to homogenize.
- 2) Prepare 10-fold serial dilutions in 0.1% peptone water.
- 3) Place a 3M Petrifilm RYM Count Plate on a flat, level surface for each dilution to be tested.
- 4) Lift the top of the film. Dispense 1mL of each dilution onto the center of the bottom film of each plate.
- 5) Roll the film down onto the sample.
- 6) Place the 3M™ Petrifilm™ Flat Spreader (Cat. No. 6425) on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Count Plate growth area before the gel is formed. **Do not slide the spreader across the film.**
- 7) Remove the spreader and leave the plate undisturbed for at least 1 minute to permit the gel to form.
- 8) Incubate the 3M Petrifilm RYM Count Plates at 25 or 28°C in a horizontal position with the clear side up in stacks of no more than 40 plates. Enumerate plates after 48 hours of incubation. If colonies appear faint, allow up to an additional 12 hours of incubation time for enhanced interpretation. 3M Petrifilm RYM Count Plates can be counted using a standard colony counter with the use of a back light or an illuminated magnifier to assist with the estimated enumeration.
- 9) Yeast colonies appear raised and small with defined edges. Colonies may appear pink/tan to blue/green in color.
- 10) Mold colonies appear flat with a dark center and diffused edges. Colonies may appear blue/green to variable upon prolonged incubation. *See Table 2014.05D* for yeast and mold appearance.
- 11) The circular growth area is approximately 30cm². Plates containing greater than 150 colonies can be either estimated or recorded as TNTC (too numerous to count). 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 the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be at user discretion.
- 12) Food samples may occasionally show interference on the 3M Petrifilm RYM Count Plates, for example:
 - Uniform blue background color (often seen from the organisms used in cultured products). These should not be counted as TNTC.
 - Intense pinpoint blue specks (often seen with spices or granulated products).
 - Report final results as colony-forming units/gram (CFU/g).
- 13) If required, colonies may be isolated for further identification by direct microscopy or biochemical analysis. Lift the top film and pick the colony from the gel.

Reference: *J. AOAC Int.* (future issue)

Posted: January 20, 2015

Table 2014.05A

Interlaboratory study results of 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate Method vs FDA-BAM and ISO 21527 methods for frozen raw ground beef patties

| 3M™ Petrifilm™ RYM Count Plate Method | | | | | | FDA-BAM/ISO 21527 Methods ^a | | | | | p-Value ^d | Difference of Means | Reverse Transformed Mean Difference ^e |
|---|---------|----------------|-------------------|-------------------|----------------|--|-------|-------|----------------|----------------|----------------------|---------------------|--|
| Matrix | Lot | N ^b | Mean ^c | S _r | S _R | Lot | N | Mean | S _r | S _R | | | |
| Frozen Raw Ground Beef Patties (25°C, 48 h) | Control | 11(0) | <1.00 | — | — | Control | 11(0) | <1.00 | — | — | — | — | — |
| | Low | 11(0) | 2.12 | 0.41 | 0.41 | Low | 11(1) | 2.07 | 0.36 | 0.38 | 0.5323 | 0.05 | 14.34 |
| | Medium | 11(0) | 3.52 | 0.10 | 0.10 | Medium | 11(0) | 3.47 | 0.09 | 0.11 | 0.1637 | 0.05 | 360.10 |
| | High | 11(0) | 4.65 | 0.13 | 0.14 | High | 11(0) | 4.59 | 0.10 | 0.14 | 0.2266 | 0.06 | 5763.84 |
| Frozen Raw Ground Beef Patties (25°C, 60 h) | Control | 11(0) | <1.00 | — | — | Control | 11(0) | <1.00 | — | — | — | — | — |
| | Low | 11(0) | 2.14 | 0.36 ^f | 0.37 | Low | 11(1) | 2.07 | 0.36 | 0.38 | 0.3773 | 0.07 | 20.55 |
| | Medium | 11(0) | 3.52 | 0.10 | 0.10 | Medium | 11(0) | 3.47 | 0.09 | 0.11 | 0.1573 | 0.05 | 360.10 |
| | High | 11(0) | 4.65 | 0.14 | 0.15 | High | 11(0) | 4.59 | 0.10 | 0.14 | 0.1750 | 0.06 | 5763.84 |
| Frozen Raw Ground Beef Patties (28°C, 48 h) | Control | 11(0) | <1.00 | — | — | Control | 11(0) | <1.00 | — | — | — | — | — |
| | Low | 11(0) | 2.17 | 0.29 ^f | 0.30 | Low | 11(1) | 2.07 | 0.36 | 0.38 | 0.1391 | 0.10 | 30.42 |
| | Medium | 11(0) | 3.53 | 0.10 | 0.10 | Medium | 11(0) | 3.47 | 0.09 | 0.11 | 0.0824 | 0.06 | 437.23 |
| | High | 11(0) | 4.67 | 0.08 ^f | 0.11 | High | 11(0) | 4.59 | 0.10 | 0.14 | 0.0966 | 0.08 | 7869.00 |
| Frozen Raw Ground Beef Patties (28°C, 60 h) | Control | 11(0) | <1.00 | — | — | Control | 11(0) | <1.00 | — | — | — | — | — |
| | Low | 11(0) | 2.16 | 0.29 ^f | 0.29 | Low | 11(1) | 2.07 | 0.36 | 0.38 | 0.1843 | 0.09 | 27.05 |
| | Medium | 11(0) | 3.53 | 0.09 | 0.10 | Medium | 11(0) | 3.47 | 0.09 | 0.11 | 0.1095 | 0.06 | 437.23 |
| | High | 11(0) | 4.67 | 0.08 ^f | 0.11 | High | 11(0) | 4.59 | 0.10 | 0.14 | 0.1088 | 0.08 | 7869.00 |

Table 2014.05B

Interlaboratory study results of 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate Method vs FDA-BAM and ISO 21527 methods for raw almonds

| 3M™ Petrifilm™ RYM Count Plate Method | | | | | | FDA-BAM/ISO 21527 Methods ^a | | | | | p-Value ^d | Difference of Means | Reverse Transformed Mean Difference ^e |
|---------------------------------------|---------|----------------|-------------------|-------------------|----------------|--|-------|-------|----------------|----------------|----------------------|---------------------|--|
| Matrix | Lot | N ^b | Mean ^c | S _r | S _R | Lot | N | Mean | S _r | S _R | | | |
| Raw Almonds (25°C, 48 h) | Control | 12(0) | <1.00 | — | — | Control | 12(0) | <1.00 | — | — | — | — | — |
| | Low | 14(0) | 1.45 | 0.17 ^f | 0.26 | Low | 14(0) | 1.55 | 0.19 | 0.34 | 0.4165 | 0.10 | -7.30 |
| | Medium | 14(1) | 2.12 | 0.26 | 0.39 | Medium | 14(0) | 2.21 | 0.20 | 0.24 | 0.3322 | 0.09 | -30.36 |
| | High | 14(2) | 3.00 | 0.18 | 0.49 | High | 14(1) | 3.08 | 0.12 | 0.31 | 0.2833 | 0.08 | -202.26 |
| Raw Almonds (25°C, 60 h) | Control | 12(0) | <1.00 | — | — | Control | 12(0) | <1.00 | — | — | — | — | — |
| | Low | 14(0) | 1.53 | 0.23 | 0.28 | Low | 14(0) | 1.55 | 0.19 | 0.34 | 0.8391 | 0.02 | -1.60 |
| | Medium | 14(0) | 2.20 | 0.21 | 0.27 | Medium | 14(0) | 2.21 | 0.20 | 0.24 | 0.7789 | 0.01 | -3.69 |
| | High | 14(2) | 3.04 | 0.18 | 0.41 | High | 14(1) | 3.08 | 0.12 | 0.31 | 0.5418 | 0.04 | -105.79 |
| Raw Almonds (28°C, 48 h) | Control | 12(0) | <1.00 | — | — | Control | 12(0) | <1.00 | — | — | — | — | — |
| | Low | 14(0) | 1.58 | 0.16 ^f | 0.21 | Low | 14(0) | 1.55 | 0.19 | 0.34 | 0.7381 | 0.03 | 2.54 |
| | Medium | 14(0) | 2.17 | 0.17 ^f | 0.29 | Medium | 14(0) | 2.21 | 0.20 | 0.24 | 0.6139 | 0.04 | -11.73 |
| | High | 14(2) | 3.01 | 0.17 | 0.45 | High | 14(1) | 3.08 | 0.12 | 0.31 | 0.3904 | 0.07 | -178.97 |
| Raw Almonds (28°C, 60 h) | Control | 12(0) | <1.00 | — | — | Control | 12(0) | <1.00 | — | — | — | — | — |
| | Low | 14(0) | 1.60 | 0.17 ^f | 0.20 | Low | 14(0) | 1.55 | 0.19 | 0.34 | 0.5474 | 0.05 | 4.33 |
| | Medium | 14(0) | 2.21 | 0.17 ^f | 0.23 | Medium | 14(0) | 2.21 | 0.20 | 0.24 | 0.9483 | 0.00 | 0.00 |
| | High | 14(2) | 3.03 | 0.18 | 0.42 | High | 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 Log₁₀ 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

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 |

Table 2014.05D: Appearance of yeast and mold on 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plates

| Yeast | Mold |
|--|--|
| Small colonies | Large colonies |
| Colonies have defined edges | Colonies have diffused edges |
| Pink/tan to blue/green in color | Blue/green to variable upon prolonged incubation |
| Colonies appear raised (3-dimensional) | Colonies appear flat |
| Colonies have a uniform color | Colonies have a dark center with diffused edges |



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