## 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 matrixes: raw almonds and dehydrated soup.]

*Caution*: After use, the diluents and 3M Petrifilm RYM Count 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.

*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 for detailed results of the collaborative study [*J. AOAC Int*. **98**, 767(2015)].

#### *A. Principle*

The 3M Petrifilm Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system, which contains nutrients

**Table 2014.05A. Interlaboratory study results of 3M Petrifilm RYM versus FDA-BAM and ISO 21527 methods for frozen raw ground beef patties**

| | | 3M Petrifilm RYM method | | | | FDA-BAM/ISO 21527 methods[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Lot | *N*[b] | Mean[c] | $s_r$ | $s_R$ | *N* | Mean | $s_r$ | $s_R$ | *P*-value[d] | Difference of means | Reverse transformed mean difference[e] |
| Frozen raw ground beef patties | | | | | | | | | | | | |
| 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 |
| 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.

**Table 2014.05B. Interlaboratory study results of 3M Petrifilm RYM versus FDA-BAM and ISO 21527 methods for raw almonds**

| | | 3M Petrifilm RYM method | | | | FDA-BAM/ISO 21527 methods[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Lot | *N*[b] | Mean[c] | $s_r$ | $s_R$ | *N* | Mean | $s_r$ | $s_R$ | *P*-value[d] | Difference of means | Reverse transformed mean difference[e] |
| | | | | | | Raw almonds | | | | | | |
| 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 |
| 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.

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 h 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*

(**a**) *3M Petrifilm RYM Count Plate.*—25 plates/pouch; two pouches/box (3M Food Safety, St. Paul, MN, USA).

(**b**) *Sterile diluents.*—0.1% peptone water.

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

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

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

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

(**g**) *3M Petrifilm Flat Spreader.*

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

(**i**) *Refrigerator.*—Capable of maintaining 2–8°C, for storing the 3M Petrifilm RYM Plates.

(**j**) *L-shaped spreaders.*

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

### *C. General Instructions*

(**a**) Store unopened 3M 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% RH). Return unused 3M Petrifilm RYM Plates to the 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 (20–25°C/<60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M Petrifilm RYM Plates be stored in a freezer if the laboratory temperature exceeds 25ºC (77ºF) and/or the laboratory is located in a region where the relative humidity exceeds 60% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm RYM Plates in a sealable container.

Post-incubation 3M 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.

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

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

**Table 2014.05D. Appearance of yeast and mold on 3M Petrifilm 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 |

### *D. Sample Preparation*

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

*Dairy products*.—Pipet 11 mL or weigh 11 g of sample into 99 mL sterile 0.1% peptone water. Shake 25 times to homogenize.

*All other foods*.—Weigh out 25 g of sample from test portion into a sterile stomacher bag and dilute with 225 mL 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 1 mL 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 min 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. Enumerate plates after 48 h of incubation. If colonies appear faint, allow up to an additional 12 h 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 30 $cm^2$. 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:

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

(*b*) Intense pinpoint blue specks (often seen with spices or granulated products).

(*c*) 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.

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