



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**100401**

The AOAC Research Institute hereby certifies the method known as:

### **CompactDry YM**

manufactured by

**Shimadzu Diagnostics Corporation**

**3-24-6, Ueno**

**Taito-ku, Tokyo**

**Japan 110-8736**

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated in the applicability of the method. 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*<sup>SM</sup> 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 that reads "Scott Coates".

\_\_\_\_\_  
Scott Coates, Senior Director  
Signature for AOAC Research Institute

Issue Date                      December 22, 2023

Expiration Date                December 31, 2024

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089

Internet e-mail: [aoacri@aoac.org](mailto:aoacri@aoac.org) \* World Wide Web Site: <http://www.aoac.org>

**AUTHORS**

Original Validation: NISSUI PHARMACEUTICAL CO., LTD.  
 Modification November 2015: Shingo Mizuochi and Maria Nelson

**SUBMITTING COMPANY**

NISSUI PHARMACEUTICAL CO., LTD.  
 2-11-1, Sugamo, Toshima-Ku  
 Tokyo 170-0002  
 Japan

**CURRENT COMPANY ADDRESS**

Shimadzu Diagnostics Corporation,  
 formerly NISSUI Pharmaceutical Co.,  
 Ltd.  
 3-24-6, Ueno  
 Taito-ku, Tokyo  
 Japan 110-8736

**METHOD NAME**

Compact Dry YM

**CATALOG NUMBERS**

06746, 06747

**INDEPENDENT LABORATORY**

Original Validation  
 Japan Food Research Laboratories  
 52-1, Motoyoyogi-cho, Shibuya-ku  
 Tokyo 151-0062 **Japan**

**November 2015 Modification**

Campden BRI  
 Station Road  
 Chipping Campden  
 Gloucestershire, GL55 6LD UK

**APPLICABILITY OF METHOD**

Target organisms – Yeast and mold

Matrixes – (USDA BAM) – fresh apples, frozen blueberries, orange juice, dried banana chips, & fresh grapefruit  
**MODIFICATION NOVEMBER 2015 – ISO 21527-1:2008, 10 g samples –**  
 cooked deli turkey, fresh whole tomatoes, cheese (Wensleydale), sliced white bread, mayonnaise.

Performance claims – This is a report for Compact Dry YM according to protocol instructed by AOAC RI on August 22, 2003 for matrixes of fruit. The internal and independent validation studies compared the analytical results of the Compact Dry YM Method for enumeration of yeasts and molds to the FDA BAM Method in fruit.

**REFERENCE METHODS**

Original Validation: U.S. Food and Drug Administration (2001) Bacteriological Analytical Manual, 8<sup>th</sup> Ed., Rev. A, AOAC INTERNATIONAL, Gaithersburg, MD (2)

November 2015 Modification: ISO 21527-1:2008, Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95 (4)

**ORIGINAL CERTIFICATION DATE**

October 14, 2004

**CERTIFICATION RENEWAL RECORD**

Renewed annually through December 2024.

**METHOD MODIFICATION RECORD**

1. November 2015 Level 2
2. December 2018 Level 1
3. December 2023 Level 1

**SUMMARY OF MODIFICATION**

1. Matrix Extension approval.
2. Corporate address change.
3. Corporate name change to Shimadzu Diagnostics Corporation, updated package inserts.

Under this AOAC *Performance Tested Methods*<sup>SM</sup> License Number, 100401 this method is distributed by:

1. Hardy Diagnostics
2. R-Biopharm AG

Under this AOAC *Performance Tested Methods*<sup>SM</sup> License Number, 100401 this method is distributed as:

1. Compact Dry YM
2. Compact Dry YM

**PRINCIPLE OF THE METHOD (1)**

The test method is enumeration of yeasts and molds in food (fruit).

**DISCUSSION OF THE VALIDATION STUDY (1)**

In this AOAC Performance Tested Method<sup>SM</sup> Internal Laboratory Validation Study, the Compact Dry YM was compared to the BAM method for enumeration of yeast and mold.

As evidenced by attached data of 5 replicates for 3 levels of 5 kinds of fruit (fresh apples, frozen blueberries, orange juice, dried banana chips, and fresh grapefruit; in total n=75) through internal and independent validation study, r<sup>2</sup> as correlation coefficient for both methods was 0.9856 that indicates good correlation in the figure below (Fig.6). Confidence in the data between the three levels is observed when the mean of each of the three groups is plotted and the slope is calculated. The slope is meant very close to 1.0.

Taniwaki et al. (4) compared with the dichloran rose bengal chloramphenicol (DRBC), SimPlate and Petrifilm techniques for yeast and mold enumeration in 14 foods (orange juice, corn meal etc.). In this study, correlation coefficients of DRBC versus SimPlate and Petrifilm for recovering total yeast and mold counts from the composite of 14 foods were 0.6793 and 0.9299, respectively.

For detection of yeasts and molds for fresh apple, the mean for BAM method was higher than that for Compact Dry YM method at the low, medium and high levels of contamination. The one-way ANOVA of the both methods was no significant different.

For detection of yeasts and molds for orange juice, the mean for BAM method was higher than that for Compact Dry YM method at the low, medium and high levels of contamination. The one-way ANOVA of the both methods was no significant different.

For detection of yeasts and molds for frozen blueberries, the mean for BAM method was higher than that for Compact Dry YM method at the low and medium levels of contamination. But the mean for BAM method was lower than that for Compact Dry YM method at the high level of contamination. The one way ANOVA of the both methods was no significant different.

For detection of yeasts and molds for dried banana chips, the mean for BAM method was higher than that for Compact Dry YM method at the low and medium levels of contamination. But the mean for BAM method was lower than that for Compact Dry YM method at the high level of contamination. The one way ANOVA of the both methods was no significant different.

Growth area of Compact Dry YM (diameter of 5 cm) for yeast and mold is smaller than that of petri dish (diameter of 9 cm). The Japan Food Research Laboratories carried out the independent validation study indicate that the counting of yeast colonies for the Compact Dry YM method was difficult, especially at the medium and high levels of contamination. Growth of a large mold colony sometimes overlap each other within growth area, it is necessary to careful to count of the colony at regular intervals in this case for Compact Dry YM.

The Compact Dry YM would also bring advantages in easy to use, saving the preparation time above the agar media, reduced storage space, waste disposal and required incubator space (5). The long shelf life of the product also has benefits compared to ready prepared agar, which has a limited shelf life and therefore requires more logistical planning.

Overall, the Compact Dry YM would be a very quick and easy screening method for the enumeration of yeast and mold in fruit.

**Table 2 Compact Dry YM Inclusivity Study (Yeasts and Molds) (1)**

| Strain                              | Test number | Color reaction | Negative |
|-------------------------------------|-------------|----------------|----------|
| <i>Candida albicans</i>             | 6           | B/ LB          |          |
| <i>Candida tropicalis</i>           | 5           | B/G            |          |
| <i>Candida glabrata</i>             | 2           | B              |          |
| <i>Candida krusei</i>               | 5           | B              |          |
| <i>Debaryomyces polymorphus</i>     | 2           | B/G            |          |
| <i>Pichia fermentans</i>            | 2           | B              |          |
| <i>Rhodotorula glutinis</i>         | 2           | B              |          |
| <i>Rhodotorula mucilaginosa</i>     | 2           | B              |          |
| <i>Saccharomyces</i>                | 1           | LG             |          |
| <i>Torulasporea delbrueckii</i>     | 2           | LG/G           |          |
| <i>Zygosaccharomyces rouxii</i>     | 2           | LB/B           |          |
| <i>Aspergillus clavatus</i>         | 2           | WB             |          |
| <i>Aspergillus nidulans</i>         | 1           | BrG            |          |
| <i>Aspergillus niger</i>            | 1           | Bl             |          |
| <i>Aspergillus oryzae</i>           | 2           | BrB /W         |          |
| <i>Aspergillus parasiticus</i>      | 2           | BrB            |          |
| <i>Cladosporium cladosporioides</i> | 2           | Bl             |          |
| <i>Cladosporium herbarum</i>        | 2           | BIB            |          |
| <i>Eurotium repens</i>              | 2           | WB             |          |
| <i>Fusarium oxysporum</i>           | 2           | WB             |          |
| <i>Goetrichum candidum</i>          | 2           | WB             |          |
| <i>Monascus purpureus</i>           | 1           | OB             |          |
| <i>Penicillium italicum</i>         | 1           | YBrW           |          |
| <i>Penicillium roquefortii</i>      | 1           | WB             |          |
| <i>Rhizopus stolonifer</i>          | 2           | WB             |          |
| <i>Trichoderma viride</i>           | 2           | WB             |          |
| <i>Trichothecium roseum</i>         | 2           | WB/ WBO        |          |
| <i>Verticillium dahliae</i>         | 2           | WB             |          |
|                                     | 60          |                |          |

B:Blue , W:White, G:Green, Br:Brown, Bl:Black, Y:Yellow, O:Orange, L:Light color

**Table 4 Compact Dry YM Exclusivity Study (Non-yeast, non-mold microorganisms) (1)**

| Strain                               | Test number | Color reaction | Positive |
|--------------------------------------|-------------|----------------|----------|
| <i>Aeromonas hydrophila</i>          | 2           | -              |          |
| <i>Bacillus cereus</i>               | 2           | -              |          |
| <i>Citrobacter amalonaticus</i>      | 1           | -              |          |
| <i>Citrobacter freundii</i>          | 1           | -              |          |
| <i>Citrobacter koseri</i>            | 2           | -              |          |
| <i>Edwardsiella tarda</i>            | 1           | -              |          |
| <i>Enterobacter aerogenes</i>        | 1           | -              |          |
| <i>Enterobacter agglomerans</i>      | 1           | -              |          |
| <i>Enterobacter amnigenus</i>        | 1           | -              |          |
| <i>Enterobacter asburiae</i>         | 1           | -              |          |
| <i>Enterobacter cancerogenus</i>     | 1           | -              |          |
| <i>Enterobacter cloacae</i>          | 1           | WB             | 1        |
| <i>Enterobacter sakazakii</i>        | 1           | -              |          |
| <i>Escherichia coli</i>              | 2           | -              |          |
| <i>Escherichia coli</i> O157:H7      | 2           | -              |          |
| <i>Escherichia coli</i> O111         | 2           | -              |          |
| <i>Escherichia fergusonii</i>        | 2           | -              |          |
| <i>Escherichia hermanii</i>          | 1           | -              |          |
| <i>Hafnia alvei</i>                  | 1           | -              |          |
| <i>Klebsiella oxytoca</i>            | 1           | -              |          |
| <i>Klebsiella ozaenae</i>            | 1           | -              |          |
| <i>Klebsiella pneumoniae</i>         | 2           | -              |          |
| <i>Klebsiella terrigena</i>          | 2           | -              |          |
| <i>Kluyvera ascorbata</i>            | 2           | -              |          |
| <i>Lactobacillus lactis</i>          | 1           | -              |          |
| <i>Leclercia adecarboxylata</i>      | 1           | -              |          |
| <i>Moraxella nonliquefaciens</i>     | 1           | -              |          |
| <i>Moraxella ovis</i>                | 1           | -              |          |
| <i>Proteus mirabilis</i>             | 1           | -              |          |
| <i>Proteus vulgaris</i>              | 2           | -              |          |
| <i>Pseudomonas alcalifaciens</i>     | 2           | -              |          |
| <i>Pseudomonas aeruginosa</i>        | 1           | W              | 1        |
| <i>Pseudomonas alcaligenes</i>       | 1           | -              |          |
| <i>Pseudomonas diminuta</i>          | 2           | -              |          |
| <i>Pseudomonas fluorescens</i>       | 1           | -              |          |
| <i>Pseudomonas mendocina</i>         | 1           | -              |          |
| <i>Pseudomonas pseudoalcaligenes</i> | 1           | -              |          |
| <i>Pseudomonas putida</i>            | 1           | -              |          |
| <i>Pseudomonas stutzeri</i>          | 1           | -              |          |
| <i>Pseudomonas vesicularis</i>       | 1           | -              |          |
| <i>Rahnella aquatilis</i>            | 1           | -              |          |
| <i>Salmonella choleraesuis</i>       | 2           | -              |          |
| <i>Salmonella typhimurium</i>        | 1           | -              |          |
| <i>Serratia fonticola</i>            | 1           | -              |          |
| <i>Serratia liquefaciens</i>         | 1           | B              | 1        |
| <i>Serratia marcescens</i>           | 1           | -              |          |
| <i>Serratia odorifera</i>            | 1           | -              |          |
| <i>Shigella flexineri</i>            | 2           | -              |          |
| <i>Shigella boydii</i>               | 1           | -              |          |
| <i>Staphylococcus aureus</i>         | 3           | -              |          |
| <i>Streptococcus agalactiae</i>      | 1           | -              |          |
|                                      | 68          |                | 3        |

B: Blue, W: White, -: non growth

**Table 5. AOAC Method Comparison (Fresh apples) (1)**

| Yeast/Mold LEVEL |       | Compact Dry YM |                         | BAM methods |                         |
|------------------|-------|----------------|-------------------------|-------------|-------------------------|
|                  |       | cfu/g          | log <sub>10</sub> cfu/g | cfu/g       | log <sub>10</sub> cfu/g |
| 10-100 cfu/g     | 1     | 260            | 2.41                    | 270         | 2.43                    |
|                  | 2     | 210            | 2.32                    | 270         | 2.43                    |
|                  | 3     | 170            | 2.23                    | 200         | 2.30                    |
|                  | 4     | 230            | 2.36                    | 270         | 2.43                    |
|                  | 5     | 210            | 2.32                    | 270         | 2.43                    |
|                  | Mean  | 216            | 2.33                    | 256         | 2.41                    |
|                  | Sr    | 32.86          | 0.07                    | 31.3        | 0.06                    |
|                  | RSDr% | 15.21          | 2.90                    | 12.23       | 2.42                    |
| 100-1000 cfu/g   | 1     | 3000           | 3.48                    | 3100        | 3.49                    |
|                  | 2     | 2600           | 3.41                    | 3000        | 3.48                    |
|                  | 3     | 2700           | 3.43                    | 3100        | 3.49                    |
|                  | 4     | 3100           | 3.49                    | 3000        | 3.48                    |
|                  | 5     | 2600           | 3.41                    | 2500        | 3.40                    |
|                  | Mean  | 2800           | 3.45                    | 2940        | 3.47                    |
|                  | Sr    | 234.52         | 0.04                    | 251         | 0.04                    |
|                  | RSDr% | 8.38           | 1.04                    | 8.54        | 1.13                    |
| 1000-10000 cfu/g | 1     | 24000          | 4.38                    | 23000       | 4.36                    |
|                  | 2     | 24000          | 4.38                    | 25000       | 4.40                    |
|                  | 3     | 24000          | 4.38                    | 24000       | 4.38                    |
|                  | 4     | 24000          | 4.38                    | 27000       | 4.43                    |
|                  | 5     | 22000          | 4.34                    | 26000       | 4.41                    |
|                  | Mean  | 23600          | 4.37                    | 25000       | 4.40                    |
|                  | Sr    | 894.43         | 0.02                    | 1581.14     | 0.03                    |
|                  | RSDr% | 3.79           | 0.39                    | 6.32        | 0.63                    |

**Table 7. AOAC CDYM Method Comparison (Frozen blueberries) (1)**

| Yeast/Mold LEVEL |       | cfu/g   | Compact Dry YM          |         | BAM methods             |  |
|------------------|-------|---------|-------------------------|---------|-------------------------|--|
|                  |       |         | log <sub>10</sub> cfu/g | cfu/g   | log <sub>10</sub> cfu/g |  |
| 10-100 cfu/g     | 1     | 390     | 2.59                    | 567     | 2.75                    |  |
|                  | 2     | 220     | 2.34                    | 300     | 2.48                    |  |
|                  | 3     | 460     | 2.66                    | 700     | 2.85                    |  |
|                  | 4     | 270     | 2.43                    | 333     | 2.52                    |  |
|                  | 5     | 90      | 1.95                    | 100     | 2.00                    |  |
|                  | Mean  | 286     | 2.40                    | 400     | 2.52                    |  |
|                  | Sr    | 145.02  | 0.28                    | 235.78  | 0.33                    |  |
|                  | RSDr% | 50.71   | 11.59                   | 58.95   | 13.05                   |  |
| 100-1000 cfu/g   | 1     | 2800    | 3.45                    | 2800    | 3.45                    |  |
|                  | 2     | 2800    | 3.45                    | 2633    | 3.42                    |  |
|                  | 3     | 1300    | 3.11                    | 1333    | 3.12                    |  |
|                  | 4     | 5300    | 3.72                    | 6433    | 3.81                    |  |
|                  | 5     | 3300    | 3.52                    | 3567    | 3.55                    |  |
|                  | Mean  | 3100    | 3.45                    | 3353.2  | 3.47                    |  |
|                  | Sr    | 1440.49 | 0.22                    | 1899.79 | 0.25                    |  |
|                  | RSDr% | 46.47   | 6.36                    | 56.66   | 7.11                    |  |
| 1000-10000 cfu/g | 1     | 26000   | 4.41                    | 24670   | 4.39                    |  |
|                  | 2     | 22000   | 4.34                    | 18670   | 4.27                    |  |
|                  | 3     | 22000   | 4.34                    | 19670   | 4.29                    |  |
|                  | 4     | 26000   | 4.41                    | 25000   | 4.40                    |  |
|                  | 5     | 27000   | 4.43                    | 24000   | 4.38                    |  |
|                  | Mean  | 24600   | 4.39                    | 22402   | 4.35                    |  |
|                  | Sr    | 2408.32 | 0.04                    | 2993.27 | 0.06                    |  |
|                  | RSDr% | 9.79    | 0.99                    | 13.36   | 1.38                    |  |

**Table 9. AOAC CDYM Method Comparison (Orange juice) (1)**

| Yeast/Mold LEVEL |       | Compact Dry YM |                         | BAM methods |                         |
|------------------|-------|----------------|-------------------------|-------------|-------------------------|
|                  |       | cfu/g          | log <sub>10</sub> cfu/g | cfu/g       | log <sub>10</sub> cfu/g |
| 10-100 cfu/g     | 1     | 210            | 2.32                    | 230         | 2.36                    |
|                  | 2     | 220            | 2.34                    | 230         | 2.36                    |
|                  | 3     | 170            | 2.23                    | 200         | 2.30                    |
|                  | 4     | 150            | 2.18                    | 130         | 2.11                    |
|                  | 5     | 160            | 2.20                    | 200         | 2.30                    |
|                  | Mean  | 182            | 2.26                    | 198         | 2.29                    |
|                  | Sr    | 31.14          | 0.07                    | 40.87       | 0.10                    |
|                  | RSDr% | 17.11          | 3.26                    | 20.64       | 4.45                    |
| 100-1000 cfu/g   | 1     | 1900           | 3.28                    | 2400        | 3.38                    |
|                  | 2     | 2100           | 3.32                    | 2300        | 3.36                    |
|                  | 3     | 2100           | 3.32                    | 2100        | 3.32                    |
|                  | 4     | 2200           | 3.34                    | 2500        | 3.40                    |
|                  | 5     | 2100           | 3.32                    | 2500        | 3.40                    |
|                  | Mean  | 2080           | 3.32                    | 2360        | 3.37                    |
|                  | Sr    | 109.54         | 0.02                    | 167.33      | 0.03                    |
|                  | RSDr% | 5.27           | 0.71                    | 7.09        | 0.94                    |
| 1000-10000 cfu/g | 1     | 20000          | 4.30                    | 25000       | 4.40                    |
|                  | 2     | 24000          | 4.38                    | 25000       | 4.40                    |
|                  | 3     | 27000          | 4.43                    | 26000       | 4.41                    |
|                  | 4     | 24000          | 4.38                    | 29000       | 4.46                    |
|                  | 5     | 25000          | 4.40                    | 25000       | 4.40                    |
|                  | Mean  | 24000          | 4.38                    | 26000       | 4.41                    |
|                  | Sr    | 2549.51        | 0.05                    | 1732.05     | 0.03                    |
|                  | RSDr% | 10.62          | 1.09                    | 6.66        | 0.63                    |

**Table 11. AOAC CDYM Method Comparison (Dried banana chips) (1)**

| Yeast/Mold LEVEL |       | Compact Dry YM |                         | BAM methods |                         |
|------------------|-------|----------------|-------------------------|-------------|-------------------------|
|                  |       | cfu/g          | log <sub>10</sub> cfu/g | cfu/g       | log <sub>10</sub> cfu/g |
| 10-100 cfu/g     | 1     | 10             | 1                       | 67          | 1.83                    |
|                  | 2     | 20             | 1.30                    | 33          | 1.52                    |
|                  | 3     | 50             | 1.70                    | 33          | 1.52                    |
|                  | 4     | 10             | 1                       | 33          | 1.52                    |
|                  | 5     | 30             | 1.48                    | 33          | 1.52                    |
|                  | Mean  | 24             | 1.30                    | 39.8        | 1.58                    |
|                  | Sr    | 16.73          | 0.30                    | 15.21       | 0.14                    |
|                  | RSDr% | 69.72          | 23.49                   | 38.20       | 8.71                    |
| 100-1000 cfu/g   | 1     | 150            | 2.18                    | 267         | 2.43                    |
|                  | 2     | 300            | 2.48                    | 300         | 2.48                    |
|                  | 3     | 260            | 2.41                    | 333         | 2.52                    |
|                  | 4     | 140            | 2.15                    | 167         | 2.22                    |
|                  | 5     | 190            | 2.28                    | 233         | 2.37                    |
|                  | Mean  | 208            | 2.30                    | 260         | 2.40                    |
|                  | Sr    | 69.79          | 0.14                    | 63.95       | 0.12                    |
|                  | RSDr% | 33.55          | 6.31                    | 24.59       | 4.84                    |
| 1000-10000 cfu/g | 1     | 2500           | 3.40                    | 2033        | 3.31                    |
|                  | 2     | 2100           | 3.32                    | 2200        | 3.34                    |
|                  | 3     | 2000           | 3.30                    | 1633        | 3.21                    |
|                  | 4     | 1600           | 3.20                    | 1667        | 3.22                    |
|                  | 5     | 1900           | 3.28                    | 1667        | 3.22                    |
|                  | Mean  | 2020           | 3.30                    | 1840        | 3.26                    |
|                  | Sr    | 327.11         | 0.07                    | 259.59      | 0.06                    |
|                  | RSDr% | 16.19          | 2.13                    | 14.11       | 1.83                    |

**Table 88. Results from enumeration of yeast and mold levels by the Compact Dry YM method and BAM (grapefruit method comparison) (1)**

| Sample code    | Counts on DRBC (BAM method) |           | Compact Dry YM method |           | Difference log between methods |
|----------------|-----------------------------|-----------|-----------------------|-----------|--------------------------------|
|                | cfu/g                       | Log cfu/g | cfu/g                 | Log cfu/g |                                |
| Low level      | 1.0×10 <sup>3</sup>         | 3.00      | 1.0×10 <sup>3</sup>   | 3.00      | 0                              |
|                | 8.7×10 <sup>2</sup>         | 2.94      | 8.5×10 <sup>2</sup>   | 2.93      | 0.01                           |
|                | 8.0×10 <sup>2</sup>         | 2.90      | 8.2×10 <sup>2</sup>   | 2.91      | -0.01                          |
|                | 9.7×10 <sup>2</sup>         | 2.99      | 8.7×10 <sup>2</sup>   | 2.94      | 0.05                           |
|                | 8.0×10 <sup>2</sup>         | 2.90      | 8.2×10 <sup>2</sup>   | 2.91      | -0.01                          |
| Medium level   | 8.5×10 <sup>3</sup>         | 3.93      | 7.8×10 <sup>3</sup>   | 3.89      | 0.04                           |
|                | 8.1×10 <sup>3</sup>         | 3.91      | 7.7×10 <sup>3</sup>   | 3.89      | 0.02                           |
|                | 8.1×10 <sup>3</sup>         | 3.91      | 7.8×10 <sup>3</sup>   | 3.89      | 0.02                           |
|                | 8.1×10 <sup>3</sup>         | 3.91      | 6.4×10 <sup>3</sup>   | 3.81      | 0.10                           |
|                | 8.2×10 <sup>3</sup>         | 3.91      | 8.0×10 <sup>3</sup>   | 3.90      | 0.01                           |
| High level     | 7.8×10 <sup>4</sup>         | 4.89      | 7.1×10 <sup>4</sup>   | 4.85      | 0.04                           |
|                | 8.9×10 <sup>4</sup>         | 4.95      | 7.5×10 <sup>4</sup>   | 4.88      | 0.07                           |
|                | 8.7×10 <sup>4</sup>         | 4.94      | 7.1×10 <sup>4</sup>   | 4.85      | 0.09                           |
|                | 7.4×10 <sup>4</sup>         | 4.87      | 7.1×10 <sup>4</sup>   | 4.85      | 0.02                           |
|                | 7.4×10 <sup>4</sup>         | 4.87      | 7.6×10 <sup>4</sup>   | 4.88      | -0.01                          |
| Uncontaminated | < 100                       | < 2.00    | < 10                  | < 1.00    | -                              |
|                | < 100                       | < 2.00    | < 10                  | < 1.00    | -                              |
|                | < 100                       | < 2.00    | < 10                  | < 1.00    | -                              |
|                | < 100                       | < 2.00    | < 10                  | < 1.00    | -                              |
|                | < 100                       | < 2.00    | < 10                  | < 1.00    | -                              |

**DISCUSSION OF THE MODIFICATION STUDY Approved November 2015 (3)**

For this matrix extension study, the Compact Dry YM was compared at 3- and 7-days incubation to ISO 21527-1 and the results from 3 days were compared to the results from 7 days (Tables 1–3). In the single laboratory matrix studies, the low recovery of colonies in the lowest contamination levels created large differences statistically, but these differences are based on 0–5 colonies/plate across five replicates per sample and are thus not practically important. When comparing the results of the Compact Dry YM at 3 days to ISO 21527-1, statistical differences were indicated in contamination Level 3 of deli turkey, Levels 3 and 4 of tomatoes, Levels 4 and 5 of white bread and Level 3 of mayonnaise. For Level 3 of the deli turkey and Level 5 of the white bread, the CIs were just outside of the acceptance range at (-0.563, -0.108) and (-0.598, -0.313) respectively, and the mean differences between the methods was <0.5 log<sub>10</sub> CFU/g. For the Compact Dry YM 7 day and ISO results, there was no longer a statistical difference between methods for Level 5 of the sliced bread, and the mean differences between methods for Level 3 of deli turkey, Levels 3 and 4 of tomatoes, Level 4 of white bread and Level 3 of mayonnaise were all now <0.5 log<sub>10</sub> CFU/g. Statistical differences between the Compact Dry YM 3 day and 7 day results were indicated for Level 1 of cheese, Level 2 of deli turkey and mayonnaise and Level 4 of sliced white bread and whole tomatoes. For the cheese, turkey and mayonnaise, the differences reflect counts of 0–4 colonies/plate and again are not practically important. For Level 4 of sliced white bread and tomatoes, the differences are significant and may reflect slower growing yeast or mold strains in these particular samples. However, no differences were seen in any other contamination levels for either matrix. All matrixes, with the exception of mayonnaise, were naturally contaminated with yeasts and molds, and it is possible that the contamination was not completely homogenous throughout the samples. For Level 4 of tomatoes in particular, the high s<sub>r</sub> value (>0.7) across all methods would indicate this. Also, outliers were found in Levels 2 and 3 in sliced white bread, Levels 1 and 2 of cheese, and Levels 2, 3 and 4 of tomatoes, but since no justifiable causes were noted for the outliers, no data were removed from the statistical analysis. Values for s<sub>r</sub> and RSD<sub>r</sub> were similar between the Compact Dry YM (3 and 7 days) and ISO 21527-1, particularly at the three highest contamination levels. In some cases the Compact Dry values were lower, and in other cases the ISO values were lower, but there did not appear to be a trend favoring either method. Across all matrixes, the r<sup>2</sup> values were ≥0.84 for between the Compact Dry YM 3 day and ISO 21527-1, ≥0.87 for the Compact Dry YM 7 day and ISO method and ≥0.96 for the Compact Dry YM 3 day and 7 day. The lowest r<sup>2</sup> values were seen in the tomatoes.

In the multi-laboratory study on orange juice, no statistical differences were found between the Compact Dry YM and the ISO 21527-1 for enumeration of yeasts and molds. Due to shipping and scheduling issues, two laboratories initiated testing one day later than the other laboratories. Because of this delay, the organizing laboratory tested a full set of samples on the intended start and on the next day as well. In addition, some of the collaborators indicated that some samples arrived frozen, and so the organizing laboratory stored the second set of samples at -18°C overnight before testing on the second day to determine if freezing would have an effect on the yeast and mold recovery. No differences were determined by ANOVA, and thus data were included from all laboratories. Across 11 data sets, the mean differences between the Compact Dry YM, at either time point, and ISO 21527-1 were less than 0.08 log<sub>10</sub> CFU/g with a CI of (0.015, 0.142) well within the (-0.5, 0.5) acceptance criterion. The s<sub>r</sub>, RSD<sub>r</sub>, s<sub>R</sub>, and RSD<sub>R</sub> were similar for each method, and the r<sup>2</sup> value was 1.0 for all comparisons.

**Table 1. Single laboratory matrix study: Compact Dry YM – 3 day vs ISO 21527-1 (4)**

| Matrix                  | Cont. level | Compact Dry YM – 3 day |                             |                               | ISO 21527-1 |                |                  | Mean diff. <sup>d</sup> | 95% CI <sup>e</sup> |                  | r <sup>2h</sup> |
|-------------------------|-------------|------------------------|-----------------------------|-------------------------------|-------------|----------------|------------------|-------------------------|---------------------|------------------|-----------------|
|                         |             | Mean <sup>a</sup>      | s <sub>r</sub> <sup>b</sup> | RSD <sub>r</sub> <sup>c</sup> | Mean        | s <sub>r</sub> | RSD <sub>r</sub> |                         | LCL <sup>f</sup>    | UCL <sup>g</sup> |                 |
| Cooked deli turkey      | 1           | 0.000                  | 0.000                       | NA                            | 0.000       | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.93            |
|                         | 2           | 0.312                  | 0.503                       | 161                           | 1.818       | 0.992          | 54.6             | -1.506                  | -2.220              | -0.791           |                 |
|                         | 3           | 3.294                  | 0.459                       | 13.9                          | 3.630       | 0.320          | 8.81             | -0.336                  | -0.563              | -0.108           |                 |
|                         | 4           | 5.642                  | 0.090                       | 1.60                          | 5.765       | 0.112          | 1.94             | -0.123                  | -0.152              | -0.094           |                 |
|                         | 5           | 6.539                  | 0.266                       | 4.07                          | 6.642       | 0.268          | 4.03             | -0.104                  | -0.172              | -0.035           |                 |
| Fresh whole tomatoes    | 1           | 0.000                  | 0.000                       | NA                            | 0.200       | 0.634          | 317              | -0.200                  | -0.654              | 0.253            | 0.84            |
|                         | 2           | 1.134                  | 0.477                       | 42.1                          | 1.741       | 0.944          | 54.2             | -0.607                  | -1.109              | -0.104           |                 |
|                         | 3           | 2.168                  | 0.338                       | 15.6                          | 1.741       | 1.259          | 72.3             | 0.427                   | -0.295              | 1.149            |                 |
|                         | 4           | 3.087                  | 0.700                       | 22.7                          | 3.824       | 0.833          | 21.8             | -0.737                  | -1.249              | -0.226           |                 |
|                         | 5           | 4.980                  | 0.076                       | 1.53                          | 5.267       | 0.152          | 2.89             | -0.286                  | -0.367              | -0.206           |                 |
| Wensleydalecheese       | 1           | 0.000                  | 0.000                       | NA                            | 0.862       | 1.117          | 130              | -0.862                  | -1.660              | -0.063           | 0.95            |
|                         | 2           | 3.568                  | 0.100                       | 2.80                          | 3.663       | 0.103          | 2.81             | -0.095                  | -0.166              | -0.023           |                 |
|                         | 3           | 4.490                  | 0.065                       | 1.45                          | 4.625       | 0.075          | 1.62             | -0.135                  | -0.171              | -0.098           |                 |
|                         | 4           | 5.684                  | 0.056                       | 0.99                          | 5.772       | 0.080          | 1.39             | -0.088                  | -0.148              | -0.028           |                 |
|                         | 5           | 7.290                  | 0.210                       | 2.88                          | 7.533       | 0.197          | 2.62             | -0.243                  | -0.305              | -0.108           |                 |
| Sliced white bread      | 1           | 0.000                  | 0.000                       | NA                            | 0.000       | 0.000          | NA               | 0.000                   | -0.000              | 0.000            | 0.96            |
|                         | 2           | 1.477                  | 0.749                       | 50.7                          | 0.820       | 1.353          | 165              | 0.657                   | -0.011              | 1.325            |                 |
|                         | 3           | 5.137                  | 0.299                       | 5.82                          | 5.234       | 0.339          | 6.48             | -0.097                  | -0.133              | -0.062           |                 |
|                         | 4           | 4.932                  | 0.392                       | 7.95                          | 5.946       | 0.446          | 7.50             | -1.013                  | -1.350              | -0.677           |                 |
|                         | 5           | 6.737                  | 0.261                       | 3.87                          | 7.192       | 0.164          | 2.28             | -0.455                  | -0.598              | -0.313           |                 |
| Mayonnaise <sup>i</sup> | 1           | 0.000                  | 0.000                       | NA                            | 0.200       | 0.634          | 317              | -0.200                  | -0.654              | 0.253            | 0.88            |
|                         | 2           | 0.839                  | 0.728                       | 86.8                          | 1.079       | 1.147          | 106              | -0.240                  | -1.195              | 0.715            |                 |
|                         | 3           | 2.241                  | 0.122                       | 5.44                          | 2.806       | 0.212          | 7.56             | -0.565                  | -0.699              | -0.432           |                 |
|                         | 4           | 3.744                  | 0.119                       | 3.18                          | 3.962       | 0.088          | 2.22             | -0.219                  | -0.286              | -0.151           |                 |
|                         | 5           | 4.750                  | 0.069                       | 1.45                          | 4.903       | 0.112          | 2.28             | -0.153                  | -0.200              | -0.105           |                 |

<sup>a</sup>Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log<sub>10</sub>[CFU/g + (0.1)]f.

<sup>b</sup>Repeatability standard deviation.

<sup>c</sup>Relative standard deviation for repeatability. NA = not applicable.

<sup>d</sup>Mean difference between the candidate and reference methods.

<sup>e</sup>Confidence interval.

<sup>f</sup>95% Lower confidence limit for difference of means.

<sup>g</sup>95% Upper confidence limit for difference of means.

<sup>h</sup>Square of correlation coefficient.

<sup>i</sup>Inoculated with *Pichia membranaefaciens* (Campden BRI code 16014) and *Penicillium chrysogenum* (CABI Bioscience, UK, 1394016). All other matrixes naturally contaminated.



**Table 2. Single laboratory matrix study: Compact Dry YM – 7 day vs ISO 21527-1 (4)**

| Matrix                  | Cont. level | Compact Dry YM – 7 day |                             |                               | ISO 21527-1 |                |                  | Mean diff. <sup>d</sup> | 95% CI <sup>e</sup> |                  | r <sup>2h</sup> |
|-------------------------|-------------|------------------------|-----------------------------|-------------------------------|-------------|----------------|------------------|-------------------------|---------------------|------------------|-----------------|
|                         |             | Mean <sup>a</sup>      | s <sub>r</sub> <sup>b</sup> | RSD <sub>r</sub> <sup>c</sup> | Mean        | s <sub>r</sub> | RSD <sub>r</sub> |                         | LCL <sup>f</sup>    | UCL <sup>g</sup> |                 |
| Cooked deli turkey      | 1           | 0.000                  | 0.000                       | NA                            | 0.000       | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.97            |
|                         | 2           | 1.249                  | 0.523                       | 41.8                          | 1.818       | 0.992          | 54.6             | -0.569                  | -1.092              | -0.047           |                 |
|                         | 3           | 3.366                  | 0.403                       | 12.0                          | 3.630       | 0.320          | 8.82             | -0.264                  | -0.516              | -0.012           |                 |
|                         | 4           | 5.685                  | 0.075                       | 1.32                          | 5.765       | 0.112          | 1.94             | -0.080                  | -0.113              | -0.047           |                 |
|                         | 5           | 6.557                  | 0.263                       | 4.01                          | 6.642       | 0.268          | 4.03             | -0.086                  | -0.155              | -0.016           |                 |
| Fresh whole tomatoes    | 1           | 0.000                  | 0.000                       | NA                            | 0.200       | 0.634          | 317              | -0.200                  | -0.654              | 0.253            | 0.87            |
|                         | 2           | 1.190                  | 0.480                       | 40.3                          | 1.741       | 0.944          | 54.2             | -0.550                  | -1.037              | -0.064           |                 |
|                         | 3           | 2.173                  | 0.333                       | 15.3                          | 1.741       | 1.259          | 72.3             | 0.433                   | -0.296              | 1.162            |                 |
|                         | 4           | 3.552                  | 0.737                       | 20.7                          | 3.824       | 0.833          | 21.8             | -0.272                  | -0.654              | 0.110            |                 |
|                         | 5           | 4.994                  | 0.073                       | 1.46                          | 5.267       | 0.152          | 2.89             | -0.272                  | -0.338              | -0.207           |                 |
| Wensleydale cheese      | 1           | 1.199                  | 0.728                       | 60.7                          | 0.862       | 1.117          | 130              | 0.338                   | -0.372              | 1.048            | 0.96            |
|                         | 2           | 3.592                  | 0.103                       | 2.87                          | 3.663       | 0.103          | 2.81             | -0.071                  | -0.138              | -0.004           |                 |
|                         | 3           | 4.520                  | 0.072                       | 1.59                          | 4.625       | 0.075          | 1.62             | -0.105                  | -0.139              | -0.070           |                 |
|                         | 4           | 5.707                  | 0.067                       | 1.17                          | 5.772       | 0.080          | 1.39             | -0.066                  | -0.132              | 0.000            |                 |
|                         | 5           | 7.300                  | 0.216                       | 2.96                          | 7.533       | 0.197          | 2.62             | -0.233                  | -0.296              | -0.169           |                 |
| Sliced white bread      | 1           | 0.000                  | 0.000                       | NA                            | 0.000       | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.97            |
|                         | 2           | 1.554                  | 0.818                       | 52.6                          | 0.820       | 1.353          | 165              | 0.734                   | 0.087               | 1.382            |                 |
|                         | 3           | 5.147                  | 0.308                       | 5.98                          | 5.234       | 0.339          | 6.48             | -0.087                  | -0.115              | -0.059           |                 |
|                         | 4           | 5.657                  | 0.306                       | 5.41                          | 5.946       | 0.446          | 7.50             | -0.289                  | -0.614              | 0.036            |                 |
|                         | 5           | 6.968                  | 0.175                       | 2.51                          | 7.192       | 0.164          | 2.28             | -0.224                  | -0.346              | -0.103           |                 |
| Mayonnaise <sup>i</sup> | 1           | 0.000                  | 0.000                       | NA                            | 0.200       | 0.634          | 317              | -0.200                  | -0.654              | 0.253            | 0.89            |
|                         | 2           | 1.322                  | 0.531                       | 40.2                          | 1.079       | 1.147          | 106              | 0.243                   | -0.578              | 1.063            |                 |
|                         | 3           | 2.388                  | 0.116                       | 4.86                          | 2.806       | 0.212          | 7.56             | -0.418                  | -0.565              | -0.270           |                 |
|                         | 4           | 3.748                  | 0.106                       | 2.83                          | 3.962       | 0.088          | 2.22             | -0.214                  | -0.278              | -0.150           |                 |
|                         | 5           | 4.768                  | 0.058                       | 1.22                          | 4.903       | 0.112          | 2.28             | -0.135                  | -0.187              | -0.082           |                 |

<sup>a</sup>Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log<sub>10</sub>[CFU/g + (0.1)]<sup>f</sup>.

<sup>b</sup>Repeatability standard deviation.

<sup>c</sup>Relative standard deviation for repeatability. NA = not applicable.

<sup>d</sup>Mean difference between the candidate and reference methods.

<sup>e</sup>Confidence interval.

<sup>f</sup>95% Lower confidence limit for difference of means.

<sup>g</sup>95% Upper confidence limit for difference of means.

<sup>h</sup>Square of correlation coefficient.

<sup>i</sup>Inoculated with *Pichia membranaefaciens* (Campden BRI code 16014) and *Penicillium chrysogenum* (CABI Bioscience, UK, 1394016). All other matrixes naturally contaminated.

**Table 3. Single laboratory matrix study: Compact Dry YM – 3 day vs Compact Dry YM – 7 day (4)**

| Matrix                  | Cont. level | Compact Dry YM – 3 day |                             |                               | Compact Dry YM – 7 day |                |                  | Mean diff. <sup>d</sup> | 95% CI <sup>e</sup> |                  | r <sup>2h</sup> |
|-------------------------|-------------|------------------------|-----------------------------|-------------------------------|------------------------|----------------|------------------|-------------------------|---------------------|------------------|-----------------|
|                         |             | Mean <sup>a</sup>      | s <sub>r</sub> <sup>b</sup> | RSD <sub>r</sub> <sup>c</sup> | Mean                   | s <sub>r</sub> | RSD <sub>r</sub> |                         | LCL <sup>f</sup>    | UCL <sup>g</sup> |                 |
| Cooked deli turkey      | 1           | 0.000                  | 0.000                       | NA                            | 0.000                  | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.97            |
|                         | 2           | 0.312                  | 0.503                       | 161                           | 1.249                  | 0.523          | 41.9             | -0.936                  | -1.428              | -0.444           |                 |
|                         | 3           | 3.294                  | 0.459                       | 13.9                          | 3.366                  | 0.403          | 12.0             | -0.072                  | -0.193              | 0.049            |                 |
|                         | 4           | 5.642                  | 0.090                       | 1.60                          | 5.685                  | 0.075          | 1.32             | -0.043                  | -0.062              | -0.024           |                 |
|                         | 5           | 6.539                  | 0.266                       | 4.07                          | 6.557                  | 0.263          | 4.01             | -0.018                  | -0.028              | -0.008           |                 |
| Fresh whole tomatoes    | 1           | 0.000                  | 0.000                       | NA                            | 0.000                  | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.97            |
|                         | 2           | 1.134                  | 0.477                       | 42.1                          | 1.190                  | 0.480          | 40.3             | -0.056                  | -0.141              | 0.029            |                 |
|                         | 3           | 2.168                  | 0.338                       | 15.6                          | 2.173                  | 0.333          | 15.3             | -0.006                  | -0.019              | 0.007            |                 |
|                         | 4           | 3.087                  | 0.700                       | 22.7                          | 3.552                  | 0.737          | 20.7             | -0.465                  | -0.912              | -0.019           |                 |
|                         | 5           | 4.980                  | 0.076                       | 1.53                          | 4.994                  | 0.073          | 1.46             | -0.014                  | -0.039              | 0.010            |                 |
| Wensleydalecheese       | 1           | 0.000                  | 0.000                       | NA                            | 1.199                  | 0.728          | 60.7             | -0.728                  | -1.720              | -0.679           | 0.96            |
|                         | 2           | 3.568                  | 0.100                       | 2.80                          | 3.592                  | 0.103          | 2.87             | -0.024                  | -0.035              | -0.013           |                 |
|                         | 3           | 4.490                  | 0.065                       | 1.45                          | 4.520                  | 0.072          | 1.59             | -0.030                  | -0.057              | -0.003           |                 |
|                         | 4           | 5.684                  | 0.056                       | 0.99                          | 5.707                  | 0.067          | 1.17             | -0.022                  | -0.036              | -0.009           |                 |
|                         | 5           | 7.290                  | 0.210                       | 2.88                          | 7.300                  | 0.216          | 2.96             | -0.010                  | -0.022              | 0.002            |                 |
| Sliced white bread      | 1           | 0.000                  | 0.000                       | NA                            | 0.000                  | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.98            |
|                         | 2           | 1.466                  | 0.749                       | 50.7                          | 1.554                  | 0.818          | 52.6             | -0.077                  | -0.156              | 0.001            |                 |
|                         | 3           | 5.137                  | 0.299                       | 5.82                          | 5.147                  | 0.308          | 5.98             | -0.010                  | -0.027              | 0.007            |                 |
|                         | 4           | 4.932                  | 0.392                       | 7.95                          | 5.657                  | 0.306          | 5.41             | -0.724                  | -1.080              | -0.369           |                 |
|                         | 5           | 6.737                  | 0.261                       | 3.87                          | 6.958                  | 0.175          | 2.51             | -0.231                  | -0.355              | -0.107           |                 |
| Mayonnaise <sup>i</sup> | 1           | 0.000                  | 0.000                       | NA                            | 0.000                  | 0.000          | NA               | 0.000                   | 0.000               | 0.000            | 0.98            |
|                         | 2           | 0.839                  | 0.728                       | 86.8                          | 1.322                  | 0.531          | 40.2             | -0.483                  | -0.850              | -0.115           |                 |
|                         | 3           | 2.241                  | 0.122                       | 5.44                          | 2.388                  | 0.116          | 4.86             | -0.148                  | -0.177              | -0.118           |                 |
|                         | 4           | 3.744                  | 0.119                       | 3.18                          | 3.748                  | 0.106          | 2.83             | -0.004                  | -0.023              | 0.013            |                 |
|                         | 5           | 4.750                  | 0.069                       | 1.45                          | 4.768                  | 0.058          | 1.22             | -0.018                  | -0.027              | -0.009           |                 |

<sup>a</sup>Mean of five replicate portions, plated in duplicate, after logarithmic transformation:  $\text{Log}_{10}[\text{CFU/g} + (0.1)]$ .

<sup>b</sup>Repeatability standard deviation.

<sup>c</sup>Relative standard deviation for repeatability. NA = not applicable.

<sup>d</sup>Mean difference between the candidate and reference methods.

<sup>e</sup>Confidence interval.

<sup>f</sup>95% Lower confidence limit for difference of means.

<sup>g</sup>95% Upper confidence limit for difference of means.

<sup>h</sup>Square of correlation coefficient.

<sup>i</sup>Inoculated with *Pichia membranaefaciens* (Campden BRI code 16014) and *Penicillium chrysogenum* (CABI Bioscience, UK, 1394016). All other matrixes naturally contaminated.

REFERENCES CITED

1. NISSUI PHARMACEUTICAL CO., LTD., Compact Dry YM, AOAC *Performance Tested Methods*<sup>SM</sup> certification number 100401.
2. U.S. Food and Drug Administration (2001) Bacteriological Analytical Manual, 8<sup>th</sup> Ed., Rev. A, AOAC INTERNATIONAL, Gaithersburg, MD
3. Mizuochi, S., and Nelson, M., Evaluation of Compact Dry YM: Matrix Extension, AOAC® *Performance Tested*<sup>SM</sup> certification number 100401. Approved November 2015.
4. ISO 21527-1:2008, *Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95*  
[http://www.iso.org/iso/iso\\_catalogue/catalogue\\_tc/catalogue\\_detail.htm?csnumber=38275](http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=38275), accessed 2010, 2011