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laboratory (rtech laboratories, St. Paul, Minnesota, USA). Results of the independent analysis are presented in Tables 2 and 3.

The Genevision system performed as well as the reference method for the detection of *Salmonella* spp. in a variety of foods. No statistically significant differences were found between the performance of the Genevision assay and reference methods except for apple juice. In this case, it was found that the lack of sensitivity was due to the culture medium rather than the ability of the PCR assay to detect the target analyte in the enriched broth. Therefore, when testing apple juice with the Genevision system, the use of Universal Pre-Enrichment Broth (UPB) is now recommended. Internal data also showed that PCR detection on samples enriched with the Genevision-recommended culture medium could be performed after just 18 hours of enrichment instead of 24 hours, without loss of sensitivity.

Finally, this study showed that the primary enrichment procedures prescribed in the reference methods could be used equally well with the Genevision PCR detection platform as the enrichment procedure recommended by the manufacturer. No statistically significant differences were found between the Genevision PCR results obtained from samples enriched in the manufacturer-recommended culture media and those enriched in the media recommended by the corresponding official method [UPB, Lactose Broth (LB), or Buffered Peptone Water (BPW)].

Consistency, Ruggedness, and Stability

The manufacturing quality of the detection kit was also addressed in a series of experiments involving measurement of the consistency between lots, ruggedness, and stability. In an experiment measuring the consistency of results between lots of kits, it was shown that the coefficient of variation of the cycle threshold (Ct) values (the first PCR cycle at which a positive fluorescent signal is observed) was as low as 1.2% between three independent lots of kits. A real-time stability study also showed that no loss in sensitivity could be observed after a storage period of 3 months at the recommended storage temperature (4°C). Furthermore, data from an accelerated stability study showed that there was no significant degradation of the PCR signal for a period of up to 4 months of storage of complete kits at 37°C. These results suggest a stability of the kits of at least 1 year at the recommended storage temperature, but the expiry date of the Genevision kits has been set at 6 months until further real-time data become available. Finally, a ruggedness study showed that the kits could successfully withstand potential deviations from the recommended procedure.

Conclusion

The Genevision kit was found to be an efficient and user-friendly assay. Observations during these studies have shown that the Genevision system is easy to use, with a minimal amount of analyst time needed for a single diagnostic test. It was also observed that the amount of time needed for processing multiple samples when working with the Genevision system did not increase proportionally with the number of samples. This could not be said of the standard USDA Food Safety and Inspection Service (FSIS) or the *Bacteriological Analytical Manual* (BAM) methods, as there appears to be a bottleneck with the processing of samples in several areas, whereas the Genevision system allows processing of up to 45 samples at once. This factor makes the ease of use of the Genevision system highly desirable over the USDA/FSIS or the BAM methodology for processing multiple samples.

Furthermore, because of Genevision's real-time PCR technology with its two levels of DNA recognition specificity (i.e., molecular primers and molecular beacon), the Genevision™ Rapid Pathogen Detection System for *Salmonella* species yields results as good as or better than the corresponding reference method, only faster and with far less hands-on involvement. ■

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NISSUI Pharmaceutical Co. Ltd.

Nissui Compact Dry YM Test
PTM Status: October 14, 2004
Certificate No.: 100401
Cat. Nos.: 06746 (40 plates) and 06747 (240 plates)

Summary of the Validated Claims

Compact Dry YM (Figure 2) is a ready-to-use test method for the enumeration of yeasts and molds in

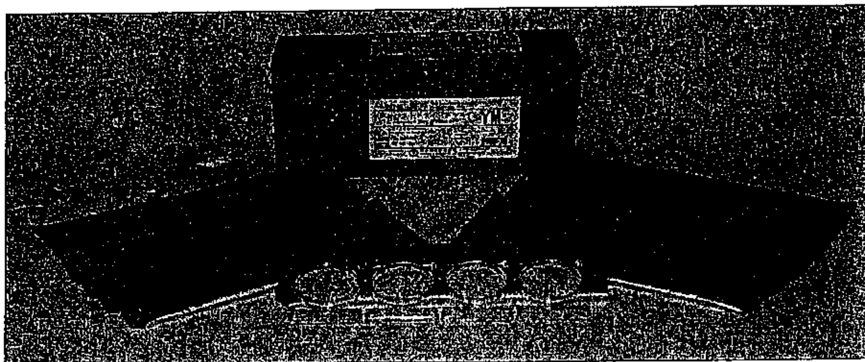

Figure 2. Compact Dry YM test kit.

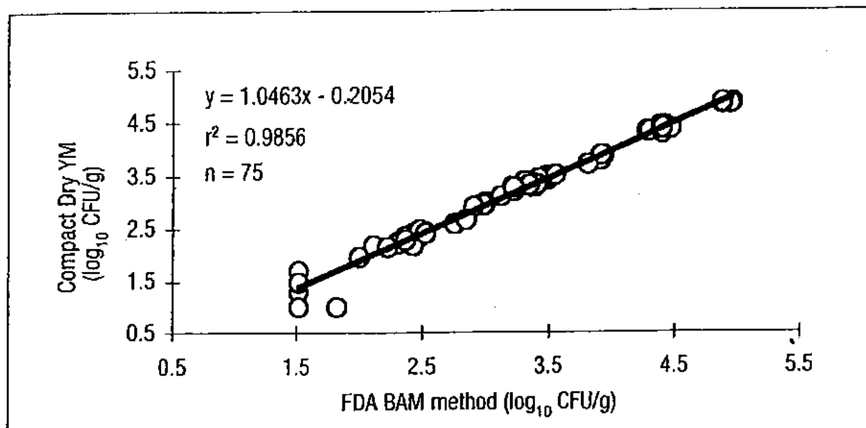

Figure 3. Accuracy study of Compact Dry YM vs the FDA BAM method.

fruit-based products. The plates are presterilized, containing nutrients supplemented with antibiotics and dyes and a cold water-soluble gelling agent. The medium should be rehydrated with 1 mL (diluted) sample material, which will diffuse automatically, and can be incubated and analyzed without any further manipulations. The plate is stackable, slim, and small—ideal for incubation and storage. The dry and sterile nature of the medium makes the plate stable for a minimum of 1 1/2 years (shelf life). The Compact Dry YM medium plates were validated as an analysis tool for determining colony forming units (CFU) of yeasts and molds from a variety of fruit-based products. Therefore, the method was validated with five different fruit products. For Compact Dry YM, 20°–25°C is the recommended incubation temperature and the standard method defined conditions. Therefore, the performance tests were conducted at 25°C. In all studies performed, no apparent differences between the Compact Dry YM method and the FDA/BAM method (http://www.cfsan.fda.gov/~ebam/bam-18.html) results were observed. For the inclusivity and exclusivity claim, the data yielded 100% (61 yeast and mold strains) and 95.6% (68 non-target strains), respectively. For the accuracy claim, correlation of the enumeration method was investigated. For all pooled sample data (n = 75), a correlation factor of $r^2 = 0.9856$ (Figure 3) could be assigned, as stated in the application for "performance tested method." The quality consistency and storage robustness of the dehydrated film plates could be demonstrated with the claimed food matrixes. No significant variations in yeast and mold counts were observed with different production lots or plates of diverse storage age (four lots; expiry before 16, 5, and 2 months and after 4 months). The Compact Dry YM plates can be used for the estimation of yeast and mold counts for a broad spectrum of fruit-based products, but because of microbial physiology the recommended optimized incubation parameters of a fixed temperature and 7 days incubation period should be kept constant. A sample volume deviation of ±10% can be tolerated, if the result is normalized to 1.0 mL for the calculation. An incubation temperature deviation from 20° to 25°C can be tolerated. The colony counts were equivalent when whole plate count and grid count (1 cm × 1 cm count × 20 and 0.5 cm × 0.5 cm count × 80) were used.

Operation of the Compact Dry YM

(*1*) Open aluminium bag and remove set of four plates.
(*2*) Detach necessary number of plate(s) from a set of four by bending up and down while pressing the lid. Use a set of four plates being connected when serial dilution measuring is intended.
(*3*) Remove cap off of the plate, pipette 1 mL sample onto the middle of the dry sheet, and replace the cap. Sample diffuses automatically and evenly throughout the sheet (total medium of 20 cm^2) to transform it into a gel within seconds.
(*4*) Write the appropriate information on the memorandum section. Turn the capped plate over and place in an incubator. Incubate 7 days at 25°C.
(*5*) From the backside of the plate, count the number of colored colonies in the medium. White paper placed under the plate makes counting colonies easier. When the number of colonies is high, it is convenient to use the grids carved on the back of the container consisting of 1 × 1 cm or 0.5 × 0.5 cm squares at the four corners. Count the colonies of one square and multiply by 20 or 80, respectively.

Features of Compact Dry YM

The Compact Dry YM is designed to be a small and compact plate, requiring only small physical spaces for storing, testing, and incubating. The Compact Dry YM is a portable plate and is ready to use (no need to prepare medium), which eliminates the waste of medium as well as the apparatus to prepare the medium. In addition, the Compact Dry YM is ideal for emergency and field test use. It allows the sample to diffuse automatically and evenly throughout the plate, with no need for mixing and diluting after sampling. Storage is easy because it is supplied as a dried plate with 1 1/2 years shelf life at room temperature. Once a liquid sample is inoculated, the dry coated medium transforms to a gel and the plate is ready to incubate. After incubation for 7 days at 25°C, the colonies on Compact Dry YM are easy to read because of blue color development by a chromogenic enzyme substrate. Isolated colonies on the Compact Dry YM can be subcultured individually to other media. No apparent differences between the Compact Dry YM and the FDA BAM method were observed in the AOAC Research Institute (RI) *Performance Tested Methods*SM (PTM) validation study.

Independent Evaluation

The Japan Food Research Laboratories conducted independent laboratory validation studies following the direction of the AOAC RI.

A study of repeatability was carried out in the independent laboratory with fresh grapefruit. Reference method was the FDA BAM method, performed exactly as specified, with no deviations

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or alterations.

As shown in Table 4, comparable values of Compact Dry YM analysis and the FDA BAM method were obtained. The standard deviation values are similar on three levels with both methods. The results from three levels by both methods are not significantly different by one-way analysis of variance (ANOVA; $p > 0.05$). This phenomenon could be observed with the other bacterial load levels and the other four matrixes (fresh apple, frozen blueberries, orange juice, and dried banana chips) tested by the internal validation study. For all remaining levels and samples by the internal validation study, the standard deviation of analogous samples delivered comparable values for Compact Dry YM as well as the FDA BAM method. And as shown in Figure 3, r^2 as correlation coefficient for both methods was 0.9856, indicating good correlation for overall levels.

Table 4. Method comparison (independent laboratory data)

Level, CFU/g	Level spiked, CFU/g	Replicate	Compact Dry YM Yeasts and molds CFU/g	Compact Dry YM Yeasts and molds log_{10}CFU/g	FDA BAM method Yeasts and molds CFU/g	FDA BAM method Yeasts and molds log_{10}CFU/g
Low level ca 10^3	7.7×10^2	1	1000	3.00	1000	3.00
		2	850	2.93	870	2.94
		3	820	2.91	800	2.90
		4	870	2.94	970	2.99
		5	820	2.91	800	2.90
		Mean	872	2.94	888	2.95
		s_r	74.63	0.04	93.65	0.05
		RSD_r, %	8.56	1.21	10.55	1.55
Medium level ca 10^4	8.2×10^3	1	7800	3.89	8500	3.93
		2	7700	3.89	8100	3.91
		3	7800	3.89	8100	3.91
		4	6400	3.81	8100	3.91
		5	8000	3.90	8200	3.91
		Mean	7540	3.88	8200	3.91
		s_r	646.53	0.04	173.21	0.01
		RSD_r, %	8.57	1.02	2.11	0.23
High level ca 10^5	7.9×10^4	1	71000	4.85	78000	4.89
		2	75000	4.88	89000	4.95
		3	71000	4.85	87000	4.94
		4	71000	4.85	74000	4.87
		5	76000	4.88	74000	4.87
		Mean	72800	4.86	80400	4.90
		s_r	2489.98	0.01	7162.40	0.04
		RSD_r, %	3.42	0.30	8.91	0.78
Uncontaminated	0	1	<10	<1.00	<100	<2.00
		2	<10	<1.00	<100	<2.00
		3	<10	<1.00	<100	<2.00
		4	<10	<1.00	<100	<2.00
		5	<10	<1.00	<100	<2.00
		Mean	<10	<1.00	<100	<2.00
		s_r	—	—	—	—
		RSD_r, %	—	—	—	—

Consequently, the repeatability of both procedures is well correlated.

Conclusions

The results obtained from the AOAC RI's PTM program for the comparison of the performance of the Compact Dry YM to that of the FDA BAM method for estimation of yeast and mold colony counts showed that these two methods performed equally well. Therefore, the Compact Dry YM could be a convenient alternative method for routine microbiological testing of fruit for yeast and mold colony counts.

The big advantages of the Compact Dry YM system are the reduced hands-on time and economical usage, as confirmed by the independent laboratory. In terms of plate preparation, inoculation, and reading the result, the Compact Dry YM system is easier and quicker than the conventional plate technique. Reading the plates is faster with the Compact Dry YM system, with the chromogenic enzyme substrate X-phos speeding up counting. It was observed that food particles, when present, did not appear to absorb the indicator. Instructions on the use of the Compact Dry YM are clear and unambiguous. The Compact Dry YM system reduces storage space, waste disposal, and required incubator space. The long shelf life of the product also has benefits compared with ready-prepared agar, which has a limited shelf life and therefore requires more logistical planning. ■

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PTM Status: (October 14, 2004)
Certificate No.: 100402

Conventional coliform counts are conducted by enumeration of colonies on VRB agar plates or by the LST broth most probable number (MPN) method as the official methods, while qualitative detection of coliforms can be achieved using BGLB broth. They require confirmation tests and take 2–5 days to obtain results. Recently, various media with chromogenic enzyme substrates have been developed. Coliform counts take only 1 day using such a chromogenic medium. However, even this test takes some hours to prepare the medium. The Sanita-kun Coliforms (Figure 4) device consists of a nonwoven fabric to which a layer of microbial nutrients is deposited in a film, similar to the Sanita-kun Aerobic Count (PTM Certificate No. 011001). The nutrient film also contains 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, or X-gal, which is hydrolyzed by β-galactosidase from coliforms to produce a visible blue dye. The nonwoven fabric and nutrient film are constructed on a base film. The basic device (fabric, nutrients, and base film) is then inserted between an adhesive backing to stabilize the basic device and a transparent film. The adhesive backing and the transparent top film serve to deter evaporation and facilitate inspection of the inoculated device. Sample solutions containing bacteria are deposited on the fabric portion of the device. Then, the solution diffuses throughout the entire pad to dissolve and release the nutrient compound film, forming a highly viscous solution. Bacteria migrate to the surface of the fabric where coliforms metabolize the nutrients and X-gal to produce a visible blue dye after incubation, while growth of noncoliforms is inhibited with selective reagents. This report details the internal validation conducted by the authors and the independent laboratory study at Silliker Inc., Research Center in comparison with the VRB agar method as a reference according to the AOAC guidelines.

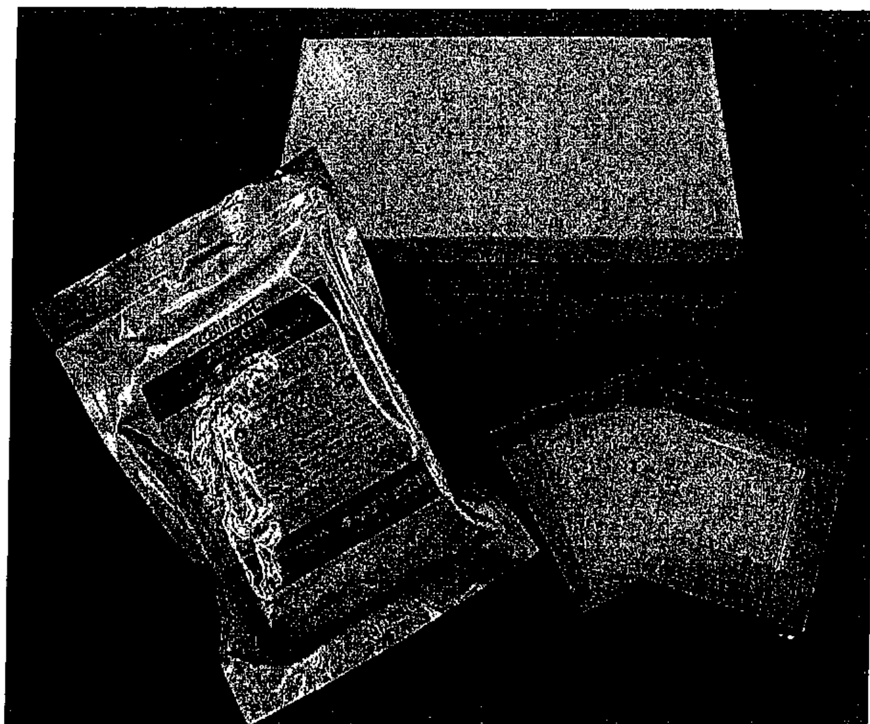

Figure 4. Sanita-kun Coliforms test kit.

Method Validation

The Sanita-kun sheet was validated by in-house and independent laboratory study using uninoculated and inoculated foods compared with VRB agar method according to FDA BAM as a reference. In a comparative study on 63 uninoculated foods, raw foods showed good agreement in colony counts with the Sanita-kun sheets and the VRB agar plates, and the linear correlation coefficient (r^2) to the VRB agar was calculated as 0.98 as shown in Figure 5, while some frozen foods and most foods frozen for 2 weeks after inoculation showed significantly higher colony counts on the Sanita-kun sheets than the VRB plates. This deposition was also observed even after confirmation of the colonies on the Sanita-kun sheets. The results of frozen and foods frozen for 2 weeks after inoculation suggested that Sanita-kun sheets

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