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Method Validation

In-House Study

The results presented in Table 1 show that the Genevision Rapid Pathogen Detection Systems for *Listeria* spp. and *L. monocytogenes* can be reliably used for the screening of the target pathogen in a variety of foods. In a first set of experiments, the inclusivity and exclusivity of the PCR primers and molecular beacon used in the *Listeria* spp. assay were investigated with a panel of 79 *Listeria* spp. strains and 288 non-*Listeria* species. All *Listeria* spp. strains were successfully detected while none of the non-*Listeria* strains reacted positively. Similarly, inclusivity and exclusivity of the *L. monocytogenes* assay were investigated with a panel of 51 *L. monocytogenes* strains and 129 non-*L. monocytogenes* strains. All *L. monocytogenes* strains were successfully detected while none of the non-*L. monocytogenes* strains reacted positively. The 100% inclusivity and 100% exclusivity rates achieved for each kit demonstrate that the PCR primers and molecular beacons used in the Genevision Rapid Pathogen Detection Systems for *Listeria* spp. and *L. monocytogenes* are extremely specific to the target organisms.

Independent Study

The performance of the two assays on artificially inoculated samples compared with traditional microbiological methods (i.e., USDA, FDA, or AOAC reference methods) was also evaluated, both internally and by an external validation laboratory (rtech laboratories, St. Paul, Minnesota, USA). Results are presented in Tables 2 and 3.

Generally, no significant differences were observed between the Genevision assays and the USDA, FDA, or AOAC reference methods except for green beans (Table 2, *L. monocytogenes*) and precut lettuce (Table 3, *Listeria* spp.) in which the Genevision system was found to be more sensitive. In these two cases, the Genevision system was able to detect a significantly greater number of confirmed, infected samples than the corresponding traditional method. There were also a number of instances where the Genevision system was able to detect the presence of the pathogen in a sample, but the result could not be confirmed microbiologically. These apparent false positives were likely due to the high level of pre-existing microflora in the particular food, which can impede isolation of the organism by traditional culture methods. Since the PCR test is largely unaffected by the same levels of background flora, it is possible to detect the presence of the target pathogen leading to a situation of an apparent false positive.

The manufacturing quality of the kits was also addressed in a series of experiments involving measurement of the consistency between lots, ruggedness, and stability. The consistency of manufacturing was examined across three lots: the coefficient of variation (CV) was 3.1%. Within each lot studied, the CV varied between 2.4 and 2.6%. These results reflect a good consistency between and within different lots of kits. In a ruggedness analysis, it was shown that the kits could successfully withstand deviations to the procedure that are likely to occur in a laboratory setting. Finally, the stability of the kits was evaluated. A real-time stability study showed no significant degradation of the PCR signal after a storage period of 3 months at 4°C. This study is currently ongoing for a period of 2 years. In an accelerated study, no significant degradation of the PCR signal was observed after an incubation period of up to 4 months at 37°C. Based on these results, the expiry date of the Genevision kits has been set at 6 months when stored under the recommended conditions (4°C), subject to revision as additional real-time data become available.

Conclusion

The Genevision kits were found to be efficient and user-friendly assays. 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 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 when 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 beacons), the Genevision Rapid Pathogen Detection Systems for *Listeria* species and *L. monocytogenes* yield results as good as or better than the corresponding reference method, only faster and with much less hands-on involvement. ■

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Nissui Pharmaceutical Co. Ltd.

Nissui Compact Dry EC Test
PTM Status: November 18, 2004
Certificate No.: 110402
Cat. Nos: 06742 (40 plates) and 06743 (240 plates)

Summary of the Validation Claims

Compact Dry EC (Figure 2) is a ready-to-use test method for the detection and identification of *Escherichia coli* and coliform bacteria in raw meat including ground pork, pork, lamb, veal, and ground beef. The plates are presterilized and contain culture medium and a

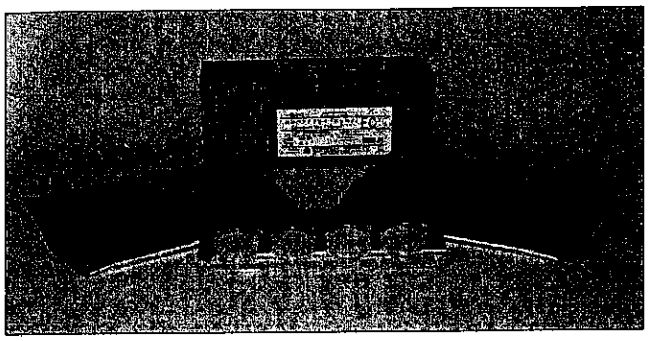

Figure 2. The Compact Dry EC.

cold water-soluble gelling agent. The medium is rehydrated with 1 mL (diluted) sample material, incubated, and analyzed without additional work steps. The plate is stackable, slim, and small—ready for storage or incubation. The dry and sterile nature makes the plate stable for a minimum of 1 1/2 years (shelf life). The Compact Dry EC medium plates were validated as an analysis tool for determining colony-forming units (CFU) of *E. coli* and coliform bacteria from a variety of raw meats using five different types of meats. The studies comparing the Compact Dry EC methodology with AOAC *Official Method*℠ **966.24** in raw meats for enumeration of *E. coli* and coliform bacteria were carried out. For Compact Dry EC, 35°C is the recommended temperature of incubation as part of the standard method defined conditions for the kit. Therefore, the performance tests were conducted at 35°C. In all studies performed, no apparent differences between the Compact Dry EC method and AOAC *Official Method*℠ **966.24** results were observed. For the accuracy claim, correlation of the enumeration method was investigated. For all pooled sample data ($n = 75$), a correlation factor of $r^2 = 0.93$ (*E. coli*) and $r^2 = 0.93$ (coliform bacteria) was obtained, as stated in the application for "performance tested method." The consistency in quality and storage robustness of the dehydrated film plates was demonstrated using the claimed food matrixes. No significant variations in *E. coli* and coliform bacterial counts were observed with different production lots or plates of diverse storage age (three lots; expiry before 11, 10, and 7 months). However, significant differences in *E. coli* and coliform bacterial counts were observed with plates stored for 40 months, beyond the expiry date of 18 months determined for the Compact Dry EC. The Compact Dry EC plates can be used for the estimation of *E. coli* and coliform bacterial counts for a broad spectrum of raw meats. However, due to microbial physiology the recommended optimized incubation parameters of 35°C and 20–24 hours should be kept constant. A sample volume deviation of 0.9 to 1.1 mL and an incubation temperature deviation from 33° to 37°C can be tolerated. The colony counts were equivalent when using whole plate count and grid count (1 cm × 1 cm count × 20 and 0.5 cm × 0.5 cm count × 80).

Operation of the Compact Dry EC

(*1*) Open aluminium bag and remove a 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 connected plates when serial dilution measuring is intended.

(*3*) Remove cap of the plate, pipet 1 mL sample in the middle of dry sheet, and replace the cap. Sample diffuses automatically and evenly into the sheet (total medium of 20 cm^2) to transform it into gel within seconds.

(*4*) Write the appropriate information on the memorandum section. Turn over the capped plate and place in an incubator. Incubate 20–24 hours at 35°C.

(*5*) From the backside of the plate, count the number of colored colonies appearing 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 EC

The Compact Dry EC is designed to be a small and compact plate that requires only small physical spaces for storing, testing, and incubating. It is portable and ready to use, with no need to prepare medium, which eliminates the waste of medium as well as the apparatus to prepare the medium. The system is especially good for emergency and field testing and samples diffuse automatically and evenly into the plate, so mixing and diluting after sampling is not required. The dried plate is easy to store with 1 1/2 years shelf life at room temperature. Once a liquid sample is inoculated, the dry coated medium transforms to gel and the plate is ready to incubate. After incubation for 20–24 hours at 35°C, the colonies on Compact Dry EC are easy to

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Table 4. Method comparison for *E.coli* (independent laboratory data)[a]

Level	Nissui	AOAC	SD	*p*
High	3.65	3.55	0.110	0.120
Intermediate	2.66	2.66	0.147	0.039
Low	1.87	1.75	0.125	0.138

[a] Mean log of actual counts of samples out of a total of five replicates for each category; standard deviation (SD) and *p* values from paired *t*-tests.

Table 5. Method comparison for coliform bacteria (independent laboratory data)[a]

Level	Nissui	AOAC	SD	*p*
High	3.72	3.61	0.103	0.128
Intermediate	2.78	2.66	0.041	0.002
Low	1.92	1.88	0.133	0.662

[a] Mean log of actual counts of samples out of a total of five replicates for each category; standard deviation (SD) and *p* values from paired *t*-tests.

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read because of a red/pink color for coliform bacteria, except for *E. coli*, and blue/blue purple color for *E. coli* due to two chromogenic enzyme substrates. The total number of coliform bacteria is the combined total number of both red/pink and blue/blue purple colonies. Isolated colonies on the Compact Dry EC can be subcultured individually to the other media. No apparent differences between the Compact Dry EC and AOAC *Official Method*℠ **966.24** were found by the AOAC Research Institute validation.

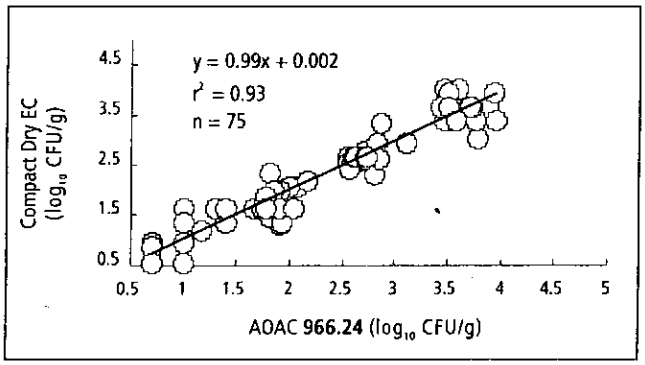

*Figure 3. Accuracy study of Compact Dry EC vs AOAC Official Method℠ **966.24** for E. coli.*

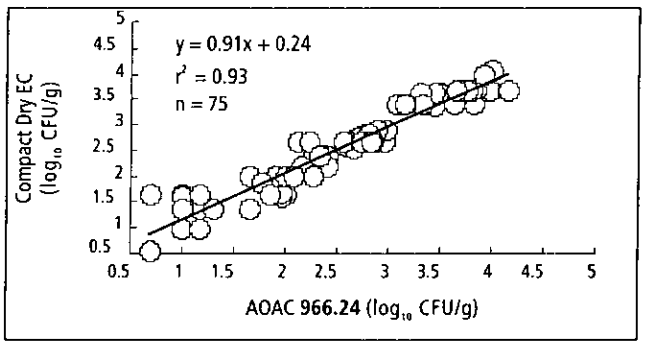

*Figure 4. Accuracy study of Compact Dry EC vs AOAC Official Method℠ **966.24** for coliform bacteria.*

Independent Evaluation

The independent laboratory validation studies were conducted by Q Laboratories, Inc., under the direction of the AOAC Research Institute.

A study of repeatability was carried out in the independent laboratory using raw ground beef. The reference method used was AOAC *Official Method*℠ **966.24**, performed exactly as specified, with no deviations or alterations.

As shown in Tables 4 and 5, comparable values for *E. coli* and coliform bacteria using Compact Dry EC analysis and AOAC *Official Method*℠ **966.24** were obtained. The Nissui EC plates yielded a consistent result for each of the five replicates at each of the three inoculum levels. The range of variance within each of the three levels was also narrower than the range from each of the results from those samples carried out according to the AOAC methodology with the exception of the intermediate level. The *p* value from paired *t*-tests for the intermediate levels showed *p* values <0.05, indicating significant difference. The difference was significant because the standard deviation (SD) of the log of coliform levels was very narrow for both methods (0.041). The mean log of *E. coli* levels, however, was exactly the same for both methods: 2.66. All five replicates for both coliform and *E. coli* yielded a result consistent with the inoculum levels expected for all five data points (460 CFU/g). The Nissui EC plates yielded coliform results ranging from 530 to 710 CFU/g. *E. coli* results ranged from 400 to 530 CFU/g. However, the results from each level with the other bacterial load and also the other four matrixes (raw ground pork, raw pork, raw lamb, and raw veal) by both methods were not significantly different by one-way analysis of variance (ANOVA; $p > 0.05$) in the internal validation study. For all remaining levels and samples in the internal validation study, the SD of analogous samples yielded comparable values for Compact Dry EC and AOAC *Official Method*℠ **966.24**. As shown in Figures 3 and 4, r^2 as correlation coefficient for both methods was 0.93 for *E. coli* and 0.93 for coliform bacteria, indicating good correlation for overall levels. Consequently, the repeatability of both procedures is comparable.

Conclusions

The results obtained from the AOAC Research Institute's *Performance Tested Method*℠ program for the comparison of the performance of the Compact Dry EC with that of AOAC *Official Method*℠ **966.24** for the detection and identification of *E. coli* and coliform bacteria showed that these two methods performed equally well. Therefore, the Compact Dry EC is a convenient alternative method for routine microbiological testing of raw meat, including ground pork, pork, lamb, veal, and ground beef for the detection and identification of *E. coli* and coliform bacteria.

The main advantages of the Compact Dry EC system are 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 EC system is easier and quicker than the conventional most probable number (MPN) technique. Reading the plates was faster with the Compact Dry EC system, with the chromogenic enzyme substrates Magenta-GAL and X-GLUC 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 EC are clear and unambiguous. Additional advantages of the Compact Dry EC system are reduced storage space, waste disposal, and required incubator space. The long shelf life of the product is also a benefit. ■

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Mention of trade names or commercial products is for identification only and does not constitute preference over similar ones not mentioned. If you are interested in submitting an article regarding your test kit that has been granted *Performance Tested Methods*℠ status, contact Deborah McKenzie at dmckenzie@aoac.org.