Evaluation of the TEMPO® EB Method for the Enumeration of *Enterobacteriaceae* in Foods

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95th Annual Meeting IAFP 2008 Columbus, Ohio · August 3–6

P3-47

ABSTRACT

Introduction: The TEMPO® EB (*Enterobacteriaceae*) method was developed for the automated enumeration of *Enterobacteriaceae* in foods. This method utilizes a selective dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the Most Probable Number. **Objective: As part of the AOAC® Research Institute validation process, the TEMPO EB method was compared to the Compendium of Methods for the Microbiological Examination of Foods for all foods**. **Methods:** Eighteen naturally and artificially contaminated foods were tested including meat, poultry, egg products, dairy, fish and seafood, and vegetables. For each food, three lots and five replicates of each lot were tested for a total of 270 samples. A 1:10 dilution of each sample was prepared and stomached for 2 min. For each diluted and stomached sample, 1.0 ml of diluted food sample was added to a TEMPO medium vial that had been reconstituted with 3.0 ml of sterile distilled water. The inoculated medium in the TEMPO vial was then transferred and sealed into the EB card by the automated TEMPO filler. The inoculated TEMPO EB cards were incubated for 22-27 h at 35 ± 1°C. Cards were read using the automated TEMPO reader. Standard method testing was performed as detailed in the Compendium. **Results:** For the majority of samples tested, there was no significant difference for both the mean log counts and repeatability between the TEMPO EB method and the standard method using a paired t-test and f-test at the 5% level. **Significance:** TEMPO EB provides an automated, accurate method for the enumeration of *Enterobacteriaceae* in foods.

MATERIALS AND METHODS

Foods

270 samples from six different food categories (18 food matrices) were enumerated by the TEMPO automated and reference plate count methods. Foods were screened for natural contamination with *Enterobacteriaceae*. When *Enterobacteriaceae* were found, three naturally contaminated lots were obtained for each food matrix. If naturally contaminated foods were not available, the food samples were inoculated with *Enterobacteriaceae* cultures at low, medium, and high levels with a log separation between each level. Five replicates of each of the three lots were analyzed for all of the food products. Raw ground pork, fresh ground beef, heat processed cooked roast beef, fresh ground chicken, frozen cooked chicken, heat processed grilled chicken, frozen catfish, heat processed frozen fish, raw cod, bagged salad, frozen green beans, hash brown potatoes, vanilla ice cream, pasteurized milk, milk powder, pasteurized eggs, rice, and dry pet food were tested in this study.

Protocols

Reference Method (Compendium): A 1:10 primary dilution was prepared for each sample. Decimal dilutions were performed to obtain results in a countable range. In duplicate, 1 mL of each dilution was transferred to violet red bile agar with glucose (VRBG) pour plates. Each plate received a VRBG overlay. The plates were incubated for 18-24 h at 35 ± 1°C (32 ± 1°C for dairy). For each plate, the number of purple-red colonies surrounded by a zone of precipitated bile acids was recorded within the counting range of 15-150 colonies. The average of the duplicate plates within the countable range was reported.

Tempo® EB Method: The method utilizes a dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the Most Probable Number. For this validation, 1 mL of a 1:10 primary dilution was transferred to a TEMPO EB medium vial previously reconstituted with 3 mL of sterile distilled water. The vial was vortexed for 3 seconds and the contents of the vial were transferred and sealed in a TEMPO EB card by the automated TEMPO filler. The TEMPO EB cards were incubated for 22-27 h at 35 ± 1°C. The TEMPO EB cards were then read using the TEMPO reader.

Figure 1. TEMPO Prep Station

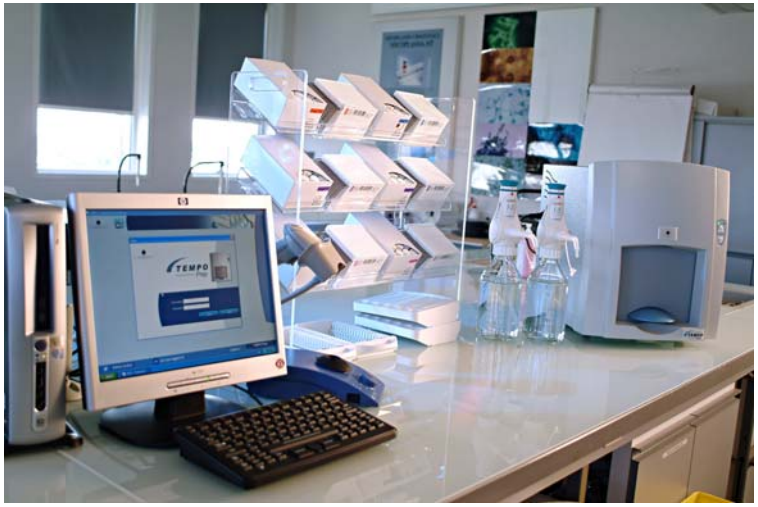
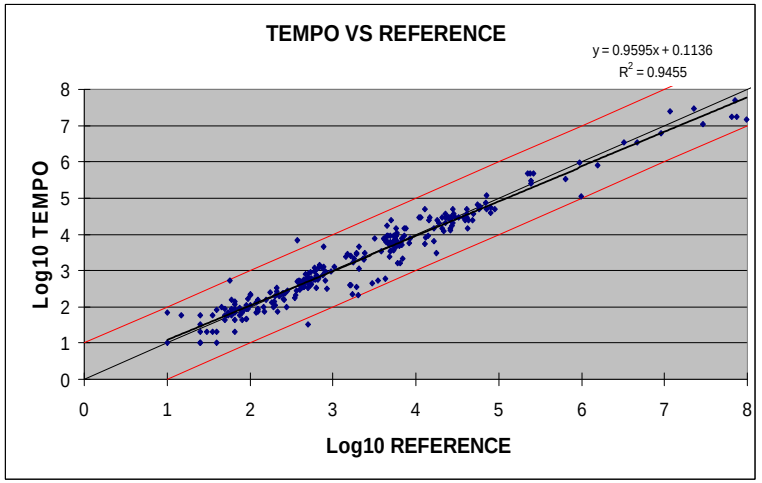


Figure 2. Regression analysis



RESULTS & DISCUSSION

The bacterial counts for both methods were transformed to $\log_{10}$ and the bias and corresponding 95% confidence interval of the bias were calculated. Regression analysis was also performed on the data set along with a t-test of the mean values for the TEMPO and the reference method. The bias, 95% confidence interval, and t-test results for the data set are detailed in Table 1.

The overall percent agreement between the TEMPO EB and reference method was calculated by determining the number of data points which exhibited log differences of less than one. **The percent agreement within one log for this study was 99%.**

Table 1. The observed bias between the TEMPO EB and Reference Method

	Bias	Confidence Interval	P value
TEMPO EB vs. Reference	-0.02	[-0.06;0.02]	0.259

The mean log counts for the TEMPO EB method on the entire data set were not significantly different from those of the reference method.

Regression analysis of this data set is detailed in Figure 2. **The two methods gave similar results with a correlation coefficient R^2=0.95, an intercept equal to 0.11 and a slope of 0.96.**

The analysis of bias yielded a confidence interval result that indicates that there is **no bias between the TEMPO EB and the reference method** for these study data.

CONCLUSIONS

- **There is no significant bias observed between the two methods evaluated in this study.**
- **The level of agreement between the two methods is 99%.**
- **The TEMPO EB method performance is equivalent to the standard method evaluated during this study.**
- **The TEMPO EB method provides an automated, accurate method for the enumeration of *Enterobacteriaceae* in foods.**
- **The TEMPO EB method has been awarded AOAC Research Institute Performance Tested Methods℠ status, Certificate No. 050801.**