



Evaluation of the TEMPO® TVC for Enumeration of Total Aerobic Flora in a Variety of Foods

T. Sofia¹, C. Ilter¹, P.J. Cotte-Pattat¹ and R. Johnson²

¹ bioMérieux, R&D Microbiologie Industrielle, Chemin de l'Orme, 69280 Marcy l'Etoile, France

² bioMérieux inc., 595 Anglum Road, Hazelwood, Saint Louis, USA.

ABSTRACT

In the food industry, Aerobic Plate Count (APC) is performed by a labor intensive plate count method. The TEMPO system was developed to improve laboratory efficiency and to replace both plate and tube enumeration methods. In this study, the TEMPO TVC (Total viable Count) method was compared to the USDA and FDA-BAM plate count method by testing a variety of food products including raw and processed meat, raw and processed poultry, fish, seafood, and vegetables. Samples were homogenized, diluted and plated in duplicate. Aliquots from the same sample dilutions were pipetted into the TEMPO TVC media reagent and automatically transferred into the enumeration card by the TEMPO filler. Plate reading was performed after a 48-hour incubation at 35°C, TEMPO TVC counts were obtained after a minimum 40-hour incubation at 35°C. Results from the two methods were compared for 140 samples using the Campden statistical guidelines for food microbiology methods. Regression analysis of log counts demonstrate good correlation between the new method and reference methods. The automated method is simpler, less time-consuming and generates less waste volume than the conventional plate count method. The TEMPO method is a valuable alternative to the conventional method.

INTRODUCTION

In the food industry, most quality indicators are determined by time-consuming plate counting methods. The automated TEMPO system has been developed to replace serial dilutions and tedious plate reading with a simple 1/10 dilution by an automated enumeration based on MPN (Most Probable Number) method. The performance of this new method was demonstrated by comparing the counts from naturally contaminated foods using both the TEMPO Total Viable Count (TVC) method and the plate count reference method (USDA and AOAC/BAM).

TEMPO SYSTEM PRINCIPLE

The system associates two single-use disposables (figure 1a) which are specific to the flora to be detected : a vial containing dehydrated culture medium and an enumeration card with 48 wells of 3 different volumes. The medium is inoculated with a 1/10 dilution of food sample which is transferred by the TEMPO Filler (figure 1b) into the card. After filling, the Filler seals the card in order to avoid contamination during handling. After card incubation, microorganism growth is detected by the TEMPO Reader (figure 1c). Depending on the number and size of the positive wells, the system calculates the number of microorganisms present in the sample.



Figure 1 : TEMPO system and reagents

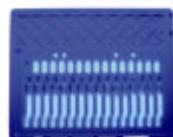


Figure 2 : TEMPO fluorescent signal after incubation

The TEMPO TVC method allows Total Viable Count enumeration from 100 to 4 900 000 CFU/g. The medium has been developed to ensure rapid growth and detection of the wide variety of food microorganisms by using a cocktail of fluorogenic substrates. During incubation, the microorganisms present in the card reduce at least one of the substrates, and cause a fluorescent signal to appear (figure 2), which is detected by the TEMPO Reader (figure 1c). Card reading and interpretation are managed by the system after only a 40-48-h incubation at 35°C.

MATERIALS AND METHODS

Foods

A total of 140 samples from 6 different food categories (Table 1) were enumerated by both automated and reference plate count method.

Food category	Examples	Total of samples
Poultry	Ground turkey, turkey sausage, ground chicken, chicken wings, turkey breast, frozen chicken breast	37
Meat	Ground beef, beef stew, ground pork, ground sirloin	33
Fish & Seafood	Sea scallops, shrimp, catfish nuggets, catfish fillet, halibut steak, tilapia fillet, cod, yellowfin tuna	20
Vegetables	Celery, salad blend, bean sprout, mushrooms, spinach, broccoli, carrots, iceberg salad, green beans	23
Prepared food	Crab cake, bean & cheese burrito, chicken Caesar salad, chicken kabob, carrot cake	8
Miscellaneous	Onions buns, jellydonuts, dry and frozen eggs, pet food	19
TOTAL		140

Protocols

Plate Count Method (USDA, AOAC/BAM) : 1 ml of primary dilution and serial dilutions up to 10⁻⁴ in Butterfield's phosphate buffer were included in PCA, 2 plates per dilution. After 48 hours incubation at 35°C, colonies were visually counted to allow total viable flora enumeration.

Automated method : either 1.0 ml or 0.1 ml of the primary dilution was transferred in a TEMPO TVC medium vial previously reconstituted with 3.0 or 3.9 ml of sterile distilled water to achieve a final volume of 4 ml. The system ensured automatic filling of the enumeration card, and after a 40-48 h incubation at 35°C, reading and MPN calculation.

Results were compared with the plate count method by regression analysis.

RESULTS

Figure 3 illustrates the regression analysis and indicates slope and intercept with 95% confidence intervals (in brackets). The regression data analysis suggests that the two methods give similar results. Less than 5 % of results (5 samples) gave a difference greater than 1 Log₁₀ between methods.

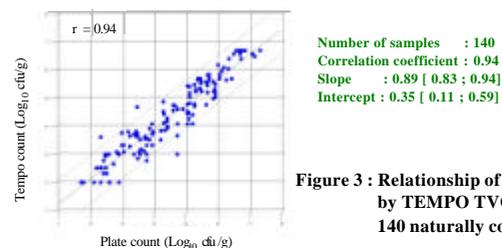


Figure 3 : Relationship of log₁₀ Total Viable Counts/g by TEMPO TVC method to PCA plates with 140 naturally contaminated food samples

CONCLUSION

TEMPO TVC showed accurate results for the enumeration of total aerobic flora in a large panel of naturally contaminated food samples. TEMPO method is easy to use and represents a real improvement from the standard methods. Automation offers a more flexible laboratory organisation as well as important economic savings in terms of labor by eliminating the need for serial dilution and the visual interpretation of results.

By providing results in less than 48 hours, TEMPO TVC offers valuable time savings and accelerates product release.