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Evaluation of the Automated TEMPO® System for Enumeration of *Escherichia coli* in Food Products

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INTRODUCTION

In food products, *Escherichia coli* levels are mostly determined by labor intensive tube and plate counting methods. The automated TEMPO® system has been developed by bioMérieux to replace serial dilutions and tedious plate preparation and reading with a simple 1/10 dilution and an automated enumeration based on MPN (Most Probable Number) method. Applied to *E. coli* enumeration, the TEMPO® EC method offers important economic savings in terms of labor as well as improved standardization, and was compared to the current standard plate count method ISO 16649-2.

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, adapted to ensure a rapid detection of microorganism growth, is inoculated with a 1/10 dilution of food sample and homogeneously transferred by the TEMPO® Filler (figure1b) into the card. After filing, 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 microorganism present in the sample.

Figure 1 : TEMPO® system and reagents

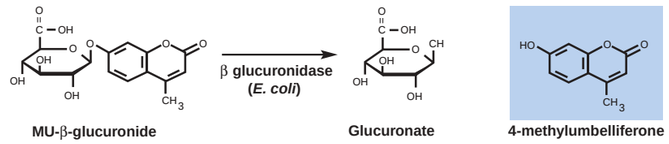
1a :TEMPO® reagents

1b : Preparation station with TEMPO® Filler on the right

1c : Reading station with TEMPO® Reader on the right

The TEMPO® EC method allows *E. coli* enumeration from 10 to 49 000 CFU/g in food sample. During incubation, the *E. coli* strains present in the card reduce the medium substrate, MU-β-glucuronide, and cause a 4-MU fluorescent signal to appear (figure 2). Card reading and interpretation are managed by the system after a 24-h incubation at 37°C.

Figure 2 : detection principle



MU-β-glucuronide **Glucuronate** **4-methylumbelliferone**

MATERIAL AND METHODS

Inclusivity and exclusivity studies :
Twenty different *E. coli* strains from culture collections and food sample origin, and twenty seven non-*E. coli* strains encountered in food products were tested (see table 1). Two *E. coli* strains were known to be β-glucuronidase negative.

Table 1 : non *E. coli* strains tested

	Bacteria / Yeast and mold*	
Aeromonas hydrophila	*Lactobacillus plantarum*	*Salmonella typhimurium*
Bacillus subtilis	*Lactococcus lactis*	*Serratia liquefaciens*
Buttiauxella agrestis	*Listeria innocua*	*Shigella sonnei*
Citrobacter freundii	*Micrococcus kristinae*	*Staphylococcus aureus*
Enterobacter cancerogenes	*Pantoea agglomerans*	*Vibrio cholerae*
Enterococcus faecalis	*Pectobacterium carotovorum*	*Aspergillus niger* *
Escherichia vulneris	*Proteus vulgaris*	*Candida tropicalis* *
Corynebacterium diphteriae	*Providencia stuartii*	*Mucor* *
Klebsiella oxytoca	*Pseudomonas aeruginosa*	*Saccharomyces cerevisiae* *

Each *E. coli* strain was serially diluted in Tryptone-salt and tested by the automated method at 2 levels : approximately 40 and 2 000 CFU per card which represents respectively 400 and 2 x 10^4 CFU/g contamination of food sample. The 10^3 suspension used to inoculate the card was controlled by traditional method (inclusion of 1 ml in Tryptone-Bile-Glucuronide (TBX) agar, incubation 18-24 hours at 44°C). Each non-*E. coli* strain was tested in the same way by traditional method, but at a higher level by the automated method : approximately 5 x 10^4 CFU per card, which represents a 5 x 10^5 CFU/g contamination of sample.

Accuracy study with food samples :
A total of 175 samples from 77 different food products were enumerated by both automated method and ISO plate count method.

Thirty six samples were chosen to represent the different good groups, in order to test the compatibility of the new method with 4 common diluents : Tryptone-salt, Buffered peptone water, Butterfield's phosphate and, for cheeses only, Sodium Citrate. Ten grams of product were diluted in 90 ml of each diluent and inoculated with *E. coli* ATCC 8739 at an approximate level of 1 000 CFU/g.

Raw and processed meat, including poultry :	22
Dairy food :	22
Fish & seafood :	7
Delicatessens and baby food :	12
Confectionary and pastry :	8
Vegetables, dried fruits and spices :	6

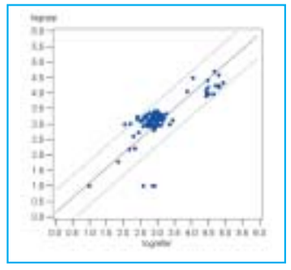
The 41 other food products were diluted in Tryptone-salt and Buffered peptone water without *E. coli* inoculation. Two of them (pork sausage and spicy sausage) were found to be naturally contaminated with *E. coli*.
ISO 16649-2 method : 1 ml of primary dilution and serial dilutions up to 10^{-3} in Tryptone-salt were included in TBX agar, 2 plates per dilution. After 18-24 h incubation at 44°C, specific blue colonies of β-glucuronidase positive strains were visually counted to allow *E. coli* enumeration.
Automated method : 1 ml of the primary dilution was transferred in a TEMPO® EC medium vial that was reconstituted with 3 ml of sterile distilled water. The system ensured automated filling of the card, and after a 24-h incubation at 37°C, reading and MPN calculation were completed.
Results from automated and ISO method were compared after conversion to the base10 logarithm by regression analysis and paired T Test of mean difference.

RESULTS

Inclusivity and exclusivity studies :
The new method detected each *E. coli* strain at the two levels except the two β-glucuronidase negative which were undetected by both methods. The ISO method failed to detect four additional strains due to incubation temperature inhibition (44°C with the plate count method versus 37°C by the new method).
Only 1 non-*E. coli* strain tested, *Shigella sonnei*, was detected by both method.

Accuracy study :
Figure 3 illustrates the **regression analysis** and indicates slope and intercept with 95% confidence intervals (in brackets). In spite of the non optimal distribution of the data set, the regression data analysis suggests that the two methods give similar results.
The different primary diluents tested did not influence the enumeration : for each product, all the primary dilutions gave similar results.
There was no false positive result due to food matrices, the automated method showed a high specificity.
The automated method failed to numerate 3 samples (see the bottom of the graph), due to fluorescence signal inhibition by the food matrix (spices). This inhibition could be overcome by sample dilution (lower sample volume transferred in *E. coli* medium).
The **paired T Test** at the 95% confidence interval [-0.10 ; 0.01], indicates that the 2 methods are not significantly different.

Figure 3 : relationship of $\log_{10}$ *E. coli* counts/g by TEMPO® EC method to TBX plates with food samples



Number of samples : 175
Correlation coefficient : 0.95
Slope : 0.96 [0.92 ; 1.01]
Intercept : 0.13 [0.01 ; 0.24]

CONCLUSION

No significant differences ($p<0.05$) were observed between the TEMPO® EC method and the ISO 16649-2 plate count method. The automated method showed accurate results for the enumeration of *E. coli* in a variety of foods with a specificity similar to that of the traditional method.

The TEMPO® EC method proved easy to use and represents an alternative for *E. coli* enumeration in food. It offers improved standardization as well as automated reading and recording of results and important economic savings in terms of labor by eliminating the need for serial dilutions.