

Evaluation of the TEMPO[®] System for Total Viable Count, Total Coliforms and *E. coli* Enumeration in Meat and Poultry Products

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INTRODUCTION

In food products, Quality Indicator counts are mostly determined by labour intensive tube and plate counting methods. The automated TEMPO system has been developed to replace serial dilutions, media preparation and plate reading with a simple 1/10 dilution and an automated enumeration based on the MPN (Most Probable Number) method. The application of the TEMPO system for Total Count, Total Coliforms and *E. coli* enumeration, was tested in comparison with the current standard plate count methods.

TEMPO SYSTEM PRINCIPLE

The TEMPO system consists of two parts : (1) a Filler; and (2) a Reader. Reagents are specific for the microbial group to be enumerated. The system uses two single-use disposables (Figure 1a) : a vial containing dehydrated culture medium and an innovative enumeration card with 48 wells of 3 different volumes. The culture medium, adapted to ensure rapid detection of microorganism growth, is inoculated with a dilution of the food sample and is then transferred into the card by the TEMPO Filler (Figure 1b). During incubation, the microbial growth causes a modification in fluorescent signal of the medium (Figure 1d) which is detected by the TEMPO Reader (Figure 1c). Depending on the number of positive wells, the system calculates the number of microorganisms present in the sample with a range of 10 to 49 000 CFU/g and 100 to 490 000 respectively for the 1/40 and 1/400 dilutions.

Figure 1 : TEMPO system and reagents



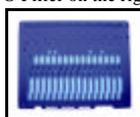
1a : TEMPO reagents



1b : Preparation station with TEMPO Filler on the right



1c: Reading station



1d: Positive card

MATERIALS AND METHODS

Samples

Fresh, frozen and dehydrated, meat, poultry and egg products were used to evaluate the following TEMPO tests : TVC (Total Viable Count), TC (Total Coliforms) and EC (*Escherichia coli*).

Table 1 : Nature of samples tested

	TVC	TC	EC
Meat products	85	85	43
Poultry products	38	30	27
Egg products	22	23	5
Total	145	138	75

Samples were collected from retail outlets. Ten grams were homogenized in 90 ml of MRD (primary 1/10 dilution) in the specific TEMPO bag with filter, and analysed by TEMPO and ISO standard methods in parallel.

Standard method protocol

1 ml of primary dilution and serial dilutions up to 10⁻³ in MRD were included in appropriate agar, 3 dilutions for Total Viable Count, 2 dilutions for Total Coliforms and *E. coli*, 2 plates per dilution. After incubation (Table 3), colonies were counted by visual inspection.

Automated method protocol

Table 2 : TEMPO media Inoculation Protocol

TEMPO Test/Dilution	Sterile Water	Inoculation Volume	Enumeration Range in CFU/g
EC and TC 1/40	3 ml	1 ml primary dilution	10 to 49.000
TC and TVC 1/400	3.9 ml	0.1 ml primary dilution	100 to 490.000
TVC 1/4000	3.9 ml	0.1 ml of a 1/100 sample dilution	1000 to 4.9 x 10 ⁶

The system ensures automated filling of the card and manages incubation time (Table 3). After incubation, the instrument reads, calculates, and prints the results.

Table 3 : TEMPO and standard method Incubation

	TEMPO Cards	Standard Method
Total Viable Count	40-48h at 30°C	ISO 4833 : PCA 72h at 30°C
Total Coliforms	24-27h at 30°C	ISO 4832 : VRBA 24h at 30°C
<i>E. coli</i>	24-27h at 37°C	ISO 16649-2 : TBX 24h at 44°C

Result analysis

Results for the automated and ISO methods were converted to Log₁₀ values.

- Analysis of agreement (difference less than 1 Log₁₀ between the 2 methods) was done separately for "in range" data (results equal to a number for both methods) and "out of range" data.
- Regression analysis was also performed.

TEMPO TVC RESULTS

Table 4 : Regression analysis TEMPO TC versus ISO 4832

"In range" data		Total data	
Number	Agreement	Number	Agreement
120	114 (95%)	289	280 (96.9%)

Agreement analysis

The rate of agreement between the two methods was very high : 97% (Table 4).

The six major discrepancies observed can be attributed to the presence of a lactic flora in the samples tested such as sausages.

The distribution of the residues (result difference between the two methods) are symmetrically distributed around 0 and the majority of result differences are within the range: [-0.3 ; 0.5] Log (Figure 2).

Regression analysis

The figure 3 suggests a very good correlation (not different from identity line Y=X), the two methods gave similar results.

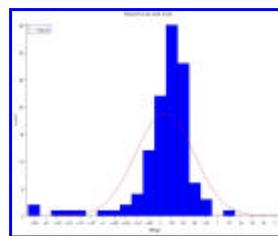


Figure 2 : TEMPO TVC versus ISO 4832 Distribution histogram of differences (in Log₁₀)

Correlation coefficient = 0,91

Intercept = 0.15 [0.074 ; 0.363] / Slope = 1.01 [0.962 ; 1.061]

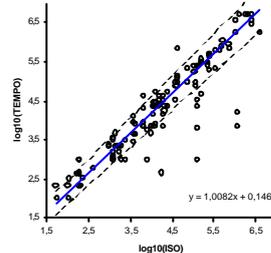


Figure 3 : Linear regression TEMPO TVC Versus ISO 4832

TEMPO EC RESULTS

Table 5 : TEMPO EC - ISO 16649-2 method agreement

"In range" data		Total data	
Number	Agreement	Number	Agreement
15	13 (86.7%)	75	69 (92%)

Agreement analysis

The rate of agreement between the two methods was high : 92%.

The 6 discrepancies concern very low level contamination : ISO enumeration <5 CFU/g and 53 to 680 CFU/g for TEMPO enumeration. The better recovery rate of the TEMPO EC versus TBX selective medium for stressed *E. coli* has been previously described in the AFNOR validation according to ISO 16140 (BIO 12/13 - 02/2005) and could explain these discrepant results.

TEMPO TC RESULTS

Table 6 : TEMPO TC - ISO 4832 method agreement

"In range" data		Total data	
Number	Agreement	Number	Agreement
172	156 (90.7%)	274	255 (93.1%)

Agreement analysis

The rate of agreement was high : 93.1% (Table 6).

The distribution of the residues (result difference between the two methods) are symmetrically distributed around 0, and the majority of result differences are within the range : [-0.7 ; 0.3] Log (Figure 4).

Regression analysis

The figure 5 suggests a very good correlation (not different from identity line Y=X), the two methods gave similar results.

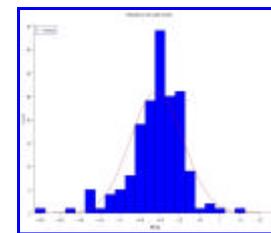


Figure 4 : TEMPO TC versus ISO 4832 Distribution histogram of differences (in Log₁₀)

Correlation coefficient = 0,89

Intercept = -0.06 [-0.265 ; 0.175] / Slope = 0.96 [0.895 ; 1.017]

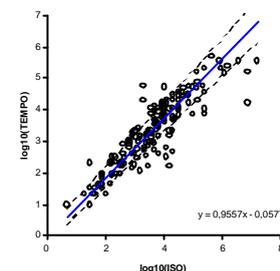


Figure 5 : Linear regression TEMPO TC Versus ISO 4832

CONCLUSION

TEMPO is a new approach to automating and simplifying enumerative tests, based on traditional and accepted MPN methods.

Studies at CCFRA have evaluated TEMPO for the enumeration of Total Viable Count, Total Coliforms and *E. coli* from meats, poultry and egg products.

Results indicate a good correlation between the ISO reference and the TEMPO results for Total Count and Coliforms and show a good rate of agreement for *E. coli* :

- TVC : 97 % of agreement ; R = 0.91
- TC : 93 % of agreement ; R = 0.89
- EC : 92 % of agreement.

TEMPO system offers important economic savings by standardizing the analysis and by minimizing the training time, the volume of waste and the number of operations.