

D. Versetti<sup>1</sup>, G. Raggi<sup>1</sup>, P. J. Cotte-Pattat<sup>2</sup>, L. Bridon<sup>2</sup>, T. Sofia<sup>2</sup>,  
<sup>1</sup>Inalca, Ospedaletto Lodigiano, ITALIA  
<sup>2</sup>bioMérieux Marcy-l'Etoile, FRANCE

## INTRODUCTION

In food products, Quality Indicator counts are mostly determined by labor intensive tube and plate counting methods. The automated TEMPO® system has been developed to replace serial dilutions, media preparation and tedious plate reading with a simple 1/10 dilution and an automated enumeration based on MPN (Most Probable Number) method. The application of the TEMPO system in an internal meat industry laboratory 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 a Filler and a Reader, the reagents are specific for the flora to be detected. The system associates 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 homogeneously transferred by the TEMPO Filler (Figure 1b) into the card. During incubation, the microorganism 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 and size of the positive wells, the system calculates the number of microorganisms present in the sample with a range of 10 to 49 000 CFU/g or 100 to 490 000 CFU/g for a 1/10 sample dilution.

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



1d : TEMPO TVC positive card

## MATERIALS AND METHODS

### Samples

A large panel of different meat samples, including raw meat, processed meat and offal, from beef, veal and pork, were used to evaluate successively the following TEMPO tests : TVC (Total Viable Count), TC (Total Coliforms) and EC (*Escherichia coli*).

Table 1 : Nature of samples tested

Number tested	TVC test		TC test		EC test
	1/400 and 1/4000 for all samples	1/40 dilution	1/400 dilution	1/40 dilution	1/40 dilution
Raw beef	50	79	15	22	
Processed beef : minced meat, hamburger, roll...	90	113	29	115	
Veal (raw and processed)	34	43	10	13	
Pork (raw and processed)	6	8	4	4	
Offal	0	26	21	1	
Total	180	269	79	155	

Samples were collected from the slaughterhouse and production line, some of them specially chosen to obtain high levels of contamination. Ten grams of these naturally contaminated products were homogenized in 90 ml of Peptone salt (primary 1/10 dilution) in the specific TEMPO bag with filter, and analysed by TEMPO and standard methods in parallel.

**Standard method protocol :** 1 ml of primary dilution and serial dilutions up to 10<sup>-3</sup> in Peptone salt were included in appropriate agar, 3 dilutions, 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	Final dilution	Medium reconstitution	Inoculation Volume	Enumeration range
EC	1/40	3 ml sterile water	+ 1 ml primary dilution	10 to 49.000 CFU/g
TC – all samples and for 79 of them	1/40	3 ml sterile water	+ 1 ml primary dilution	10 to 49.000 CFU/g
	1/400	3.9 ml sterile water	+ 0.1 ml primary dilution	100 to 490.000 CFU/g
TVC – all samples	1/400	3.9 ml sterile water	+ 0.1 ml primary dilution	100 to 490.000 CFU/g
	1/4000	3.9 ml sterile water	+ 0.1 ml of a 1/100 sample dilution	1000 to 4.9 millions CFU/g

The system ensured automated filling of the card and managed incubation time (Table 3). After incubation, the instrument read, calculated, and printed the results, preventing any transcription error.

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 : VRBL 24h at 30°C
<i>E. coli</i>	24-27h at 37°C	ISO 16649-2 : TBX 24h at 44°C

Results from the automated and ISO method were converted to the base 10 logarithm. The paired T-Test of mean difference was performed with “in range” data (results equal to a number for both methods). Analysis of agreement (difference lower than 1 log base 10 between the 2 methods) was done separately for “in range” and “out of range” data.

## TEMPO TVC RESULTS

**Agreement analysis :** the rate of agreement between the two methods was high (Table 4).

Table 4 : TEMPO TVC - ISO 4833 method Agreement

Dilution	“In range” data		Total data	
	Number	Agreement	Number	Agreement
1/400	158	150 (94,9%)	180	172 (95,5%)
1/4000	149	141 (94,6%)	177	169 (95,5%)
Total	307	291 (94,8%)	357	341 (95,5%)

Discrepant samples : Beef forequarter (2), Beef hamburger with cheese, Mini beef hamburger (2), Beef maxi burger, Vacuum pack cuts of beef (2), Veal meat, Carpaccio beef (2), Hamburger spinach, Minced beef, Veal roll.

**T-test :** The distribution of differences observed between the two methods was approximately normal (Figure 2). The bias between the two methods was -0.11 (Table 5).

Table 5 : TEMPO TVC versus ISO 4833 method T-test

Paired T-Test		0.52 [-0.17 ; -0.05] < 0.1%
Bias estimated	-0.11	
Standard deviation		
95% confident interval		
P-value of T-test		

In term of accuracy, TEMPO TVC and the reference ISO 4833 method are similar for enumeration of total viable count.

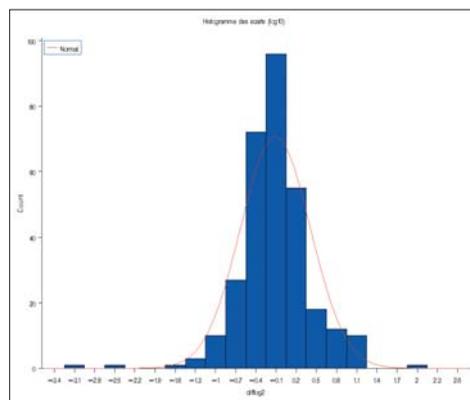


Figure 2 : TEMPO TVC versus ISO 4833 – Distribution histogram of differences in log 10.

## TEMPO TC RESULTS

**Agreement analysis :** the rate of agreement between the two methods was very high (Table 6).

Table 6 : TEMPO TC - ISO 4832 method Agreement

Dilution	“In range” data		Total data	
	Number	Agreement	Number	Agreement
1/40	189	182 (96.3%)	269	261 (97.0%)
1/400	28	28 (100%)	79	79 (100%)
Total	217	210 (96.8%)	348	340 (97.7%)

“In range” discrepant samples : Hindquarter meat, Choice minced meat, Fresh meat, Mini beef hamburger, Beef hamburger with onion, Beef Maxi burger, Portioned meat.

“Out of range” discrepant sample : Forequarter beef cuts.

**T-test :** The distribution of differences observed between the two methods was approximately normal (Figure 3). The bias between the two methods was near 0 (Table 7).

Table 7 : TEMPO TC versus ISO 4832 method T-test

Paired T –test (N=217)		0.43 [-0.04 ; +0.07] 64.7%
Bias estimated	0.013	
Ecart type		
95% confident interval		
P-value on T-test		

In term of accuracy, TEMPO TC is equivalent to the reference ISO 4832 method for enumeration of Total Coliforms.

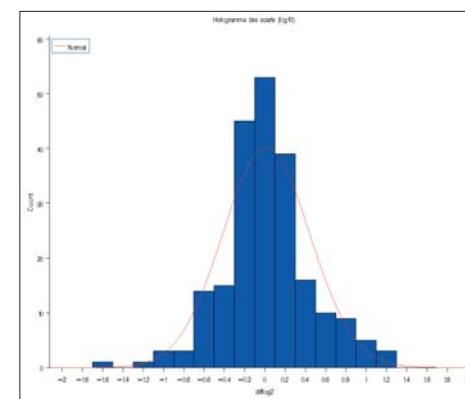


Figure 3 : TEMPO TC versus ISO 4832 – Distribution histogram of differences (in log 10).

## TEMPO EC RESULTS

In the 155 total samples, there was no discrepancy between the 2 methods.

Only 5 of them (beef heart, vacuum pack cut, beef hindquarter, beef forequarter, and fresh meat) were found naturally contaminated with *E. coli* by the two methods, with a difference lower than 0.5 log base 10 between the two results. The low number of positive samples did not allow the paired T-Test of mean difference calculation.

Sixteen additional samples were found naturally contaminated with low levels of *E. coli* (10 to 33 CFU/g) by the new method only (<10 CFU/g with the standard method). This confirms the better recovery rate of *E. coli* by this method, which was demonstrated by the TEMPO EC AFNOR validation (BIO 12/13 – 02/2005).

## CONCLUSION

The performance of the 3 TEMPO tests was equivalent to the corresponding ISO methods with very good rates of agreement. This innovative system offers improved standardization as well as important economic savings in terms of labor by eliminating serial dilutions, media preparation and tedious plate reading and represents a valuable alternative for enumeration of Quality Indicators in food industry.