



**Alternative methods for agribusiness  
Analytical performances certified**

**VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYTICAL METHOD  
ACCORDING TO STANDARD EN ISO 16140: 2003**

**Certificate No.: BIO 12/17 – 12/05**

<b>Validation date:</b>	<b>2005-12-08</b>
<b>Renewal date:</b>	<b>2009-12-04</b>
<b>End of validity:</b>	<b>2013-12-08</b>

**The company**  
(head office,  
distributor and  
production site)

**BIOMERIEUX**  
Chemin de l'Orme  
69280 MARCY L'ETOILE  
FRANCE

is hereby authorized to refer to this **AFNOR Validation certificate** for the following alternative quantitative analysis method :

**TEMPO® TC**

**Method validated for the enumeration of total coliforms in food products**

Protocol reference : 12599 version F

**SCOPE**

All human food products and pet food products, with the exception of raw milk, beverages, and cattle feed.

**RESTRICTIONS OF USE**

None.

**REFERENCE METHOD**

**ISO 4832 (2006)** - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony count technique

**Deputy General Manager  
Jacques BESLIN**

A handwritten signature in black ink, appearing to be "JBESLIN", written over a horizontal line.

**AFNOR Certification**

11, rue Francis de Pressensé – 93571 La Plaine Saint-Denis Cedex - France  
Phone +33 (0)1 41 62 80 00 – Fax +33 (0)1 49 17 90 00  
[certification@afnor.com](mailto:certification@afnor.com) - [www.afnor-validation.com](http://www.afnor-validation.com)

## PRINCIPLE OF THE METHOD

TEMPO<sup>®</sup> TC is an automated test for use with TEMPO system, for the enumeration of total coliforms in human food products and pet food products (except for products excluded from the scope)

The TEMPO system consists of 2 separate stations :

- A preparation station for inoculating the culture medium vial with the diluted food samples followed by the automated filling and sealing of the TEMPO cards by the TEMPO Filler.
- A reading station that manages the reading and interpretation of TEMPO card using the TEMPO Reader.

The culture medium contains a fluorescent substrate which, when its pH is neutral, emits a signal detected by the TEMPO Reader. During incubation, the coliforms present in the card assimilate the nutrients in the culture medium, resulting in a decrease in pH and the extinction of the fluorescent signal.

Depending on the number and size of positive wells in the card, the TEMPO system deduces the number of microorganisms present in the original sample according to a calculation based on the MPN method (Most Probable Number).

### NOTE :

In December 2009, the renewal of the validation has been pronounced without performing complementary assays since neither the TEMPO<sup>®</sup> TC method, or the reference method, nor the validated protocol has been modified.

## LINEARITY AND relative ACCURACY

### Comparison of performances of the alternative method and the reference method

#### Linearity study :

Test were performed in 2005 on the 5 food product/strain combinations and for the food categories given in the table below.

The samples were analyzed **in duplicate** by each of the **two methods**, at the 5 following artificial contamination levels : 100, 500, 1000, 5000, 50000 CFU/g.

The following results were obtained by using different dilutions (1/40, 1/400, 1/4000) :

Food category	Food product/strain pair	Regression line
Meat products	Ground beef / <i>Klebsiella oxytoca</i>	$Y = 0.072 + 0.98 X$
Pet food	Dog food / <i>Citrobacter freundii</i>	$Y = - 0.172 + 1.053 X$
Fruits & vegetables	Sliced cucumber / <i>Escherichia coli</i>	$Y = - 0.723 + 1.219 X$
Dairy products	Confectioner's custard / <i>Enterobacter agglomerans</i>	$Y = - 0.133 + 1.071 X$
Fish & sea food products	Raw fish / <i>Enterobacter cloacae</i>	$Y = - 0.562 + 1.132 X$

$Y = \log (N \text{ alternative method})$

$X = \log (N \text{ reference method})$

#### Accuracy study :

Tests were performed in 2005. The statistical interpretation was conducted on 76 results, including 72 naturally contaminated samples and 4 artificially contaminated samples, belonging to the following major food categories : meat products, pet food, fruits & vegetables, dairy products and fish & sea food products.

The samples were analyzed **in duplicate** by each of the **two methods**.

As an indication, the contamination (concentration) range were as follows :

Food category	Contamination range in log CFU/g
Meat products	1.00 to 4.63
Pet food	1.00 to 5.43
Fruits & vegetables	1.00 to 7.11
Dairy products	1.52 to 5.48
Fish & sea food products	1.40 to 5.04

The equation of the regression line between the alternative method and the reference method for all categories, is as follows :

$$Y = 0.975 X - 0.25$$

Y = log (N alternative method)

X = log (N reference method )

The repeatability for both methods and the bias between the two methods were determined according to the method of calculation used for the interlaboratory study (see sections 6.3.5 and 6.3.6 of the standard EN ISO 16140). These results provide additional information for the accuracy criterion.

The limits of repeatability (in log) obtained for the alternative method and the reference method are as follows :

Alternative Method	Reference Method
r = 0.440	r = 0.176

The bias (in log) between the two methods (alternative method - reference method) is as follows:

**D = - 0.19** by using the median, or

**D = - 0.30** by using the average of individual bias.

This bias is acceptable.

### **Conclusion for linearity and relative accuracy**

Linearity and accuracy studies show that results obtained with the TEMPO<sup>®</sup> TC method are comparable with the results obtained with the reference method EN ISO 4832, even if the repeatability limit for the alternative method is higher than the one obtained with the reference method.

## **SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)**

### **Use of alternative method only**

- 26 strains of coliform bacteria were detected on 30 tested. The 4 non detected strains were :
  - 1 *Enterobacter sakazakii*
  - 1 *Escherichia hermanii* (lactose negative)
  - 2 *Hafnia alvei* (lactose negative)

Based on the definition of coliform bacteria as lactose positive *Enterobacteriaceae*, the non detection of the 3 lactose negative strains by the alternative method was expected.

- The test of 30 non coliform bacteria has shown the detection of the 5 following strains :
  - 3 *Salmonella*,
  - 1 *Lactobacillus paracasei*,
  - 1 *Proteus mirabilis*.

From the 30 non coliform bacteria, 11 strains have shown characteristic colonies onto VRBL agar, including the 5 detected strains with the TEMPO method.

The TEMPO TC method shows a better specificity and selectivity than the reference method.

## PRACTICABILITY

### Use of alternative method only

- **Response time :**  
Results (positive or negative) are obtained in 24 hours (1 day) with both methods (reference and TEMPO TC).
- **The major interests of the TEMPO TC method are :**
  - **Important labour savings for the analysis and the reading steps:**  
With TEMPO only 4 minutes are needed to analyse one sample, in a series of 20, versus 10 minutes with the reference method.
  - **Savings in training time:**  
It takes less than one day to train an operator on a TEMPO<sup>®</sup> TC test.
  - **Space savings during incubation of the TEMPO<sup>®</sup> TC cards as well as the facilitated management of waste:**  
In order to analyse 20 samples, a rak of 20 TEMPO cards measures 22.5 x 10.2 cm. The reference method needs to use 120 plates if 20 stacks of 6 plates
  - **Complete traceability of the analysis** assured from the sample preparation station until results are delivered by the TEMPO Reader.

## INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2005 with 12 participating laboratories. The analyses were carried out on samples of half-skimmed pasteurized milk artificially contaminated with an *E. coli* strain at the 4 following levels :

- Level 0 : < 10 CFU/ml
- Level 1 : 100 to 1,000 CFU/ml
- Level 2 : 1,000 to 10,000 CFU/ml
- Level 3 : 10,000 to 100,000 CFU/ml

The laboratories tested, using each of the **two methods, two replicates per contamination level.**

The following results were obtained :

Contamina- -tion level	Number of laboratories taken into account*	Reference method		Alternative method					
		Repeatability r	Reproducibility R	Repeatability r		Reproducibility R		Bias	
				Dil 1/40	Dil 1/400	Dil 1/40	Dil 1/400	Dil 1/40	Dil 1/400
Level 1	12	0.226	0.274	0.458	/	0.500	/	-0.05	/
Level 2	12	0.161	0.183	0.277	0.371	0.392	0.550	0.08	-0.16
Level 3	12	0.144	0.157	0.319	0.279	0.445	0.308	0.16	0.02

**Conclusion**

The inter-laboratory study shows that the results with the alternative method TEMPO® TC are comparable to those obtained with the reference method. Although repeatability limit and reproducibility limit of the alternative method are higher than the reference method's ones.

Please send any queries concerning the performance of the validated method to AFNOR Certification.

You may download a summary document on the preliminary and inter-laboratory studies on [www.afnor-validation.com](http://www.afnor-validation.com)