



**Alternative methods for agribusiness  
Analytical performances certified**

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

**Certificate No.: BIO 12/15 – 09/05**

<b>Validation date :</b>	<b>2005.09.19</b>
<b>Renewal date :</b>	<b>2009.07.03</b>
<b>Extension date :</b>	<b>2009.09.24</b>
<b>End of validity :</b>	<b>2013.09.19</b>

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

**BIOMERIEUX  
69280 MARCY L'ETOILE  
FRANCE**

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

**TEMPO® TVC**

**Method validated for the enumeration of viable aerobic mesophilic flora in food products**

Protocol reference: **Réf. 80 007 - 12600 version G**

**SCOPE**

All food products for consumption by humans and domestic animals, with the exception of raw milk, beverages, and cattle feed.

**RESTRICTIONS FOR USE**

None.

**REFERENCE METHOD**

**EN ISO 4833** (Feb. 2003) : Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms – Colony count technique at 30°C.

A handwritten signature in black ink, appearing to read "Beslin", written over a white background.

**Deputy General Manager  
Jacques BESLIN**

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**AFNOR Certification**

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## PRINCIPLE OF THE METHOD

TEMPO TVC is an automated test for use with TEMPO, for the enumeration of viable aerobic mesophilic flora in food products (except for the 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.

During incubation of the card, the microorganisms present in the card reduce the substrates in the culture medium and cause a fluorescent signal to appear, which is detected by the TEMPO reader. 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 (history of validation)

1/ In July 2009, the renewal of the validation has been pronounced without performing complementary assays since neither the TEMPO TVC method, or the reference method, nor the validated protocol has been modified.

2/ In the context of the **extension study of September 2009**, new assays were realised in order to validate the storage of the TEMPO cards at 2-8°C for 48 hours maximum before reading. The results obtained after 24 hours and 48 hours of refrigeration were comparable to those obtained without refrigeration. The datas are not detailed in this certificate.

## LINEARITY AND relative ACCURACY

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

#### Linearity study:

Tests 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** with each of the **two methods**, at the five following artificial contamination levels: 100, 500, 1000, 5000, 10000 CFU/g.

The following results were obtained:

Food category	Food product/strain pair	Regression line
Meat products	Porc pâté / <i>Enterobacter agglomerans</i>	$y = 0.141 + 1.018 x$
Pet food	Cat food / <i>Bacillus cereus</i>	$y = - 0.398 + 1.137 x$
Fruits & vegetables	Green beans / <i>Xanthomonas maltophilia</i>	$y = - 0.282 + 1.101 x$
Dairy products	Confectioner's custard / <i>Staphylococcus aureus</i>	$y = 0.023 + 1.012 x$
Fish & sea food products	Raw salmon / <i>Listeria seeligeri</i>	$y = - 0.136 + 1.046 x$

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

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

#### Accuracy study:

Tests were performed in 2005. The statistical interpretation was conducted on 50 results coming from naturally 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** with each of the **two methods**.

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

Food category	Contamination range in log CFU/g
Meat products	2.23 to 6.57
Pet food	1.00 to 5.36
Fruits & vegetables	1.95 to 4.64
Dairy products	1.00 to 4.70
Fish & sea food products	1.32 to 6.32

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

$$y = 1.030 x - 0.230 \quad (40 \text{ hours of incubation})$$

$$y = 1.036 x - 0.178 \quad (48 \text{ hours of incubation})$$

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

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

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
$r = 0.528$	$r = 0.235$

The bias (in log) between the two methods (alternative method - reference method) is between **-0.08 and 0.04 log CFU/g**, in function of incubation time. This bias is not significant.

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

Linearity and accuracy studies show that results obtained with the TEMPO<sup>®</sup> TVC method are comparable with the results obtained with the reference method NF EN ISO 4833, even if the repeatability limit for the alternative method is higher than the one obtained with the reference method.

## **PRACTICABILITY**

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

- **Time to results:** Positive and negative results are obtained in 40 hours with the alternative method as opposed to 72 hours with the reference method.
- **Important labour savings at the analysis and the reading steps:** With TEMPO only 4 minutes are needed to analyse one sample, in a series of 20, versus 14 minutes with the reference method.
- **Savings in training time:** It takes less than one day to train an operator on a TEMPO<sup>®</sup> TVC test.

- **Space savings during incubation of the TEMPO® TVC cards as well as the facilitated management of waste:** The volume of disposable used with the TEMPO® TVC method is negligible compared to the volume of disposable needed for the ISO 4833 method.
- **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 13 participating laboratories. The analyses were carried out on samples of UHT milk artificially contaminated with an *Enterobacter cloacae* strain at the 4 following levels:

- Level 0 : < 10 CFU/ml
- Level 1 : 100 to 1000 CFU/ml
- Level 2 : 1000 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:

Contamination level	Number of samples taken into account*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	9	0.445	0.580	0.621	0.621	0.10
Level 2	9	0.182	0.345	0.458	0.690	0.09
Level 3	8**	0.08	0.288	0.473	0.514	0.20

*\*The results of 3 laboratories have not been taken into account because the analyses were performed out of delay*

*\*The results of a fourth laboratory have not been taken into account because the procedure for the reference method has not been respected*

*\*\*One laboratory has not given results for the level 3.*

### Conclusion

The limits of repeatability and reproducibility of the TEMPO TVC method are slightly higher than those obtained with the reference method, but remain similar.

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)