



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO 12/21 – 12/06

Validation date : 15.12.2006
End of validity : 15.12.2010

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® EB

Method validated for the enumeration of *Enterobacteriaceae* in food products

Protocol Reference: 12596 version E

SCOPE

All human food products and pet foods, excluding beverages and cattle feeds.

RESTRICTIONS OF USE

None

REFERENCE METHOD

ISO 21528-2 (August 2004): Microbiology of food and animal feeding stuffs - horizontal method for detection and enumeration of *Enterobacteriaceae* - Part 2 : colony-count method.

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 - www.afnor-validation.com

PRINCIPLE OF THE METHOD

TEMPO EB is an automated test for use with the TEMPO system for the enumeration of *Enterobacteriaceae* in human food products and pet foods (except the products excluded from its scope of application).

The TEMPO system is composed of two distinct work stations:

- A preparation station for inoculation with distribution of a diluted food sample into a bottle of culture medium, then filling and sealing of TEMPO cards with TEMPO Filler.
- A reading station for the reading and interpretation of TEMPO cards with the TEMPO Reader.

The culture medium contains a fluorescent substrate which emits a signal detected by the TEMPO Reader at neutral pH. During incubation, the *Enterobacteriaceae* present in the card assimilate nutrients in the culture medium leading to a fall in pH and suppression of the fluorescent signal. According to the number and size of the positive wells, the TEMPO system calculates the number of *Enterobacteriaceae* present at baseline in the sample according to a calculation based on the MPN (Most Probable Number) method.

LINEARITY and Relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were carried out in 2006 on 5 food product/strain combinations and for the food categories given in the table below.

Samples were analyzed **in duplicate** by each of the **two methods**, at the following five levels of artificial contamination: 100 – 500 - 1,000 - 5×10^3 - 1×10^4 CFU/g

The following results were obtained, with implementation of combined dilutions (1/40. 1/400. 1/4000):

Food category	Food product/strain combination	Regression line
Meat products	Minced beef / <i>Enterobacter cloacae</i>	Y = 1.114 X - 0.351
Egg products	Raw egg product/ <i>Klebsiella pneumoniae</i>	Y = 1.045 X - 0.091
Dairy products	Milk / <i>Escherichia coli</i>	Y = 1.102 X - 0.181
Seafood and vegetables products	Fish pâté / <i>Citrobacter freundii</i>	Y = 1.096 X - 0.299
Pet food	Dog food / <i>Hafnia alvei</i>	Y = 1.004 X - 0.012

Y = log (N alternative method)

X = log (N reference method)

Study of accuracy:

Tests were carried out in 2006. Statistical analysis was conducted on 63 interpretable results all of naturally contaminated samples belonging to the following main categories of foods:

Meat products, pet food, egg products, dairy products, seafood and vegetables products.

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

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

Food category	Range of contamination (log CFU/g)
Meat products	2.08 to 6.52
Pet foods	3.36 to 5.69
Egg products	1.54 to 6.08
Dairy products	2.70 to 6.40
Seafood and vegetables products	1.40 to 5.98

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

$$\text{Equation of line: } Y = 1.020 X - 0.037$$

Y = log (N alternative method)

X = log (N reference method)

The repeatability for the two methods and the bias between the two methods were determined according to the method of calculation used for the collaborative study (Cf. §6.3.5 and §6.3.6 of standard EN ISO 16140). These results provide additional information for the accuracy criterion. The repeatability limits obtained for the alternative method and the reference method are as follows:

Alternative method
r = 0.382

Reference method
r = 0.205

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

D= 0.050 for the mean of individual biases. This bias is non-significant.

Conclusion for linearity and relative accuracy:

The linearity and accuracy studies show that the results obtained with the TEMPO EB method are similar to the results obtained with reference method EN ISO 21528-2, though the repeatability limit was higher with the alternative method than for the reference method (except for the egg products category).

SELECTIVITY (INCLUSIVITY / EXCLUSIVITY)

Implementation of alternative method only

- 29 *Enterobacteriaceae* strains were detected out of the 30 tested. The non-recognized was a strain of *Rhanella aquatilis*, which did not respond either on VRBG medium (reference method) or by the TEMPO EB method.
- Moreover one *Citrobacter diversus* strain did not develop on VRBG but was counted by the TEMPO EB method and another *Citrobacter diversus* strain was counted by the two methods.
- The study of 21 stains not belonging to the *Enterobacteriaceae* genus showed positive reactions with two strains of *Xanthomonas maltophilia* both by the alternative and reference methods.

Conclusion

The TEMPO EB method has a satisfactory specificity.

PRACTICABILITY

- **Response time: Positive and negative** results are obtained in 24 hours with the alternative method versus 72 hours for the reference method.
- **Significant time saving both for analysis and reading:** with TEMPO only 4 minutes are required for the analysis of a sample derived from run of 20 versus 19 minutes with the reference method.
- **Reduced training time:** less than one day is required to train an operator to use the TEMPO EB test.
- **Saving in space for incubating TEMPO cards and simplified waste management:** The volume of consumables for the TEMPO EB method is negligible compared to the volume required for the ISO 21528-2 method.
- **Complete traceability of the analysis** ensured from the sample preparation station up to the analysis of results by the TEMPO Reader.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 12 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a strain of *Escherichia coli* at the 4 following levels of contamination:

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

The laboratories tested, using **both methods, 2 replicate samples** for each level of contamination. The following results were obtained:

Contamination level	Number of laboratories giving exploitable results*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	8	0.398	0.456	0.480	0.480	-0.06
Level 2	9	0.133	0.202	0.565	0.569	0.06
Level 3	9	0.166	0.226	0.160	0.397	0.08

* Four laboratories were excluded for level 1 (1 for failure to respect the protocol and 3 for a reception temperature higher than 8.4°C) and three laboratories for levels 2 and 3 (for a reception temperature higher than 8.4°C)

Conclusion

The bias between the TEMPO® EB method and the reference method gave low values of between – 0.09 and + 0.08, which were not significant.

The limits of repeatability of the TEMPO® EB method were between 0.588 and 0.160; those of the reference method were between 0.488 and 0.105.

The limits of reproducibility of the TEMPO® EB method were between 0.588 and 0.397; those of the reference method between 0.512 and 0.173.

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