



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: BIO 12/13 – 02/05

Validation date : 2005-02-04
Extension date : 2007-05-24
Renewal date : 2009-01-26
End of validity : 2013-02-04

The company
(head office, distribution, 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® EC

Method validated for the enumeration of *Escherichia coli* in food products

Protocol reference: 12597 version H

SCOPE

All human food products and pets food products, excluding raw milk, beverage, and cattle feed products.

RESTRICTIONS OF USE

None.

REFERENCE METHOD

ISO 16649-2 (April 2001): Horizontal method for the enumeration of β -glucuronidase positive *Escherichia coli* - Part 2: Colony-count technique at 44°C using 5-bromo-4chloro-3-indonyl β -D-glucuronate.

Deputy General Manager
Jacques BESLIN

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

AFNOR Certification

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PRINCIPLE OF THE METHOD

TEMPO EC is an automated test for use with TEMPO system, allowing the enumeration of *Escherichia coli* in 24 hours in food products. TEMPO EC consists of a vial of culture media and a card, which are specific to the enumeration of *E. coli*.

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.

During incubation, *E. coli* present in the card reduce the substrate 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, TEMPO system deduces the number of *E. coli* present in the original sample according to a calculation based to MPN method (Most Probable Number).

VALIDATION HISTORY

NOTE 1

In May 2007, a specific protocol was developed for milk powders including the secondary diluent MOPS buffer instead of distilled water (Cf. manufacturer's package insert for the exact procedure). This modification made it necessary to conduct complementary tests by a third party on milk powder matrix. The results of these tests are given in the **relative accuracy** section.

NOTE 2

In January 2009, the renewal of TEMPO[®]EC method has been validated. Since the previous validation, the method did not be modified and the reference method did not change. No supplementary tests were realized.

LINEARITY and Relative ACCURACY

Comparison of performances of the alternative method and reference method

Linearity study:

Tests were carried out in 2004 by artificially inoculating 5 food products with a strain of *Escherichia coli* from food, isolated from a sausage of Toulouse (see table below).

Samples were analyzed **in duplicate** by each of **the two methods**, at the five following levels of artificial contamination: 10 to 50, 50 to 100, 100 to 500, 500 to 1 000, 1 000 to 10 000 CFU/g.

The following results were obtained:

Food category	Food product /strain	Regression line	
Meat products	Minced beef / <i>E.coli</i>	$y = 0,51 + 0,89x$	N=10 R=0,96
Pet food	Food for cat / <i>E.coli</i>	$y = 0,40 + 1,17x$	N=10 R=0,97
Fruits & vegetables	Minced carrots / <i>E.coli</i>	$y = - 0,03 + 1,07x$	N=10 R=0,96
Dairy products	Confectioner's custard / <i>E.coli</i>	$y = 0,47 + 0,91x$	N=10 R=0,99
Seafood	Frozen fish / <i>E.coli</i>	$y = - 0,05 + 1,14x$	N=10 R=1,00

$y = \log$ (N alternative method)

$x = \log$ (N reference method)

Accuracy study:

Tests were carried out in 2004. Statistical analysis was conducted on 50 results including 25 naturally contaminated samples, and 25 artificially contaminated, belonging to the main following categories of foods: meat products, pet food, fruits and vegetables, dairy products and seafood products.

The 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, in log CFU/g
Meat products	1,92 to 4,44
Pet food products	1,74 to 3,92
Fruits & vegetables	1,00 to 4,18
Dairy products	1,39 to 4,31
Seafood products	1,39 to 3,22

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

$$y = 0,99 x + 0,35$$

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

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

Repeatability for the two methods and the bias between the two methods were determined according to the mode of calculation method used for the collaborative study (Cf. § 6.3.5 and § 6.3.6 of standard EN ISO 16140). These results provide complementary 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

$$r = 0,52$$

Reference method

$$r = 0,176$$

Bias (in log) between the two methods (alternative method and reference method) is as follows:

$$D = 0,26$$

Complementary tests carried out in 2007 on milk powders with the specific protocol:

Tests were performed with the new "milk powders" protocol on 30 samples contaminated by stressed strains, with a reading after 24-27 hours of incubation.

The equation of the regression line between the TEMPO EC method and the reference method, for this "milk powder" samples category was as follows:

$$Y = 1,09 x - 0,20$$

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

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

The repeatability for the two methods and the bias between the two methods were determined using the method of calculation specified above.

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

Alternative method

$$r = 0,352$$

Reference method

$$r = 0,176$$

The bias (in log) between the two methods (alternative method - reference method) is **D = 0.05**.

This bias is non-significant.

Conclusion for linearity and relative exactitude

Linearity and accuracy studies show that the results obtained with the TEMPO EC method are similar to the results obtained with the reference method EN ISO 16649-2, with a better recovering of *E. coli* with the alternative method.

For milk powders, the use of MOPS buffer rather than distilled water was better for the enumeration of *E. coli* with TEMPO EC.

SELECTIVITY (INCLUSIVITY / EXCLUSIVITY)

Implementation of the alternative method only

- Out of the 30 strains of *E. coli* tested, 28 strains producing characteristic colonies with the reference method are also detected by TEMPO EC. The 2 strains of *E. coli* (β -glucuronidase negative) producing non characteristic colonies on TBX are not detected by TEMPO EC.
- With pure strains, result of enumeration by TEMPO EC is closer to the one obtained on non-selective medium (PCA) than to the one obtained on selective medium TBX, which confirms the better recovering of *E. coli* strains with TEMPO EC method compared to the reference method on TBX medium.
- The study of 20 non- *E. coli* strains, distributed among 19 bacterial species, did not highlight any cross reactions.

PRACTICABILITY

Implementation of the alternative method only

- **Response time:** Results (positive or negative) are obtained in 1 day, with the 2 methods (reference and TEMPO EC).
- **The major interest of the TEMPO EC method consist in :**
 - **saving of important time for both analysis and reading :**
With TEMPO only 4 minutes are required to analyse one sample, out of a series of 20, versus 11 minutes with the reference method.
 - **saving of time of training :**
It takes less than one day to train an operator on a TEMPO EC test.
 - **saving space during incubation of the TEMPO EC cards and the facilitated management of waste :**
To analyze 20 samples, one support of 20 TEMPO cards measures 22,5 cm X 10,5 cm; the reference method, implies the realization of 120 plates, that is to say 20 piles of 6 Petri dishes.
 - **the complete traceability of the analysis** assured from the preparation station of samples until results were delivered by the TEMPO reader.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2004 with 12 participating laboratories. The analysis were carried out on samples of half-skimmed pasteurized milk artificially contaminated by a strain of *E. coli* 94 isolated from raw milk, at the following 4 levels :

- 0 CFU/ml
- 10 to 100 CFU/ml
- 100 to 1,000 CFU/ml
- 1,000 to 10,000 CFU/ml

The laboratories have tested, by each of the **two methods, two replicates by level** of contamination.

The following results are obtained for a 1/40 dilution of TEMPO EC:

Contamination level (CFU/ml)	Number of exploited results*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
10 to 100	22*	0,47	0,37	0,82	0,72	0,02
100 to 1,000	24	0,15	0,18	0,32	0,29	0,14
1,000 to 10,000	24	0,22	0,39	0,32	0,41	0,31

(*)Samples of one laboratory have not been exploited because there was probably an inversion for a sample between level 0 and level 1 during the realisation of the reference method.

Conclusion

The inter-laboratory study shows that the results with the alternative method are comparable with the results obtained by the reference method, with a better recovering of *E. coli* with the TEMPO® EC method.

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