



Alternative methods for agribusiness  
Analytical performances certified

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

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

**RAPID'E. COLI 2**

VALIDATED FOR THE ENUMERATION OF COLIFORMS AT 37°C

Protocol reference : RAPID'E.coli 2 / Agar (355-5299 / 356-4024) – V5

**SCOPE**

All food products for human consumption.

**RESTRICTIONS FOR USE**

None.

**REFERENCE METHOD**

**Standard NF EN ISO 4832** : Horizontal method for the enumeration of coliforms - Colony-count technique.

Deputy General Manager  
Jacques BESLIN

## PRINCIPLE OF THE METHOD

The principle of the Rapid'*E.coli* 2 medium is based on the simultaneous detection of two enzyme activities: Beta-D-Glucuronidase (GLUC) and Beta-D-Galactosidase (GAL). The medium contains two chromogenic substrates:

- one GAL-specific substrate inducing blue coloration of colonies positive for this enzyme.
- one GLUC-specific substrate inducing pink coloration of colonies positive for this enzyme.

Coliforms other than *E.coli* (GAL+/GLUC-) form blue colonies, *E.coli* (GAL+/GLUC+) form purple to pink colonies.

## LINEARITY AND relative ACCURACY

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

#### Linearity study:

Tests were performed in 2004 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: 10 to 50, 50 to 100, 100 to 500, 500 to 1000, 1000 to 10,000 CFU/g.

The following results were obtained:

Food category	Food product/strain pair	Regression line (reference method at 30°C)	Regression line (reference method at 37°C)
Meat products	Ground beef/ <i>Enterobacter agglomerans</i> source ham	$y = -0.076 + 1.020 x$	$y = -0.052 + 1.096 x$
Dairy products	Raw milk/ <i>Enterobacter cloacae</i> source powdered milk	$y = -0.151 + 1.022 x$	$y = -0.171 + 1.120 x$
Vegetable products	Red cabbage/ <i>Citrobacter freundii</i> source celery	$y = -0.089 + 1.019 x$	$y = 0.010 + 0.991 x$
Seafood products	Fish fillet/ <i>Enterobacter aminigenus</i> source fish skewer	$y = -0.009 + 0.988 x$	$y = -0.059 + 1.007 x$
Cakes and pastries	Confectioner's custard/ <i>Enterobacter cloacae</i> source raw milk	$y = 0.017 + 1.000 x$	$y = 0.040 + 0.986 x$

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

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

#### Accuracy study:

Tests were performed in 2004. The statistical interpretation was conducted on 50 results, including 43 naturally contaminated samples and 7 artificially contaminated samples, belonging to the following major food categories: meat products, dairy products, vegetable products, seafood products, cakes and pastries.

The samples were analyzed **in duplicate** with each of the **two methods** (at 37°C with Rapid'*E.coli* 2 and at 30°C and 37°C with the reference method).

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

Food category	Contamination range (in log CFU/g)
Meat products	1.9 to 4.0
Dairy products	1.9 to 4.7
Vegetable products	2.2 to 4.7
Seafood products	1.9 to 5.2
Cakes and pastries	2.5 to 4.8

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

**With the reference method at 30°C:**

$$y = -0.210 + 0.987 x$$

$$R^2 = 0.973$$

**With the reference method at 37°C:**

$$y = -0.041 + 0.981 x$$

$$R^2 = 0.985$$

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

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

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

$$r = 0.18$$

Reference method at 30°C

$$r = 0.14$$

Reference method at 37°C

$$r = 0.11$$

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

With respect to the reference method at 30°C:

$$D = -0.010 \log \text{ CFU/g}$$

With respect to the reference method at 37°C:

$$D = -0.007 \log \text{ CFU/g}$$

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

The linearity and accuracy studies demonstrate that the results obtained with the alternative method are comparable to the results obtained with the reference method.

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

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

- 67 coliform strains were detected with a characteristic appearance, out of 69 tested. The 2 strains which produced white (non-characteristic) colonies are *Hafnia alvei* strains which are OPNG-negative. These strains were tested in parallel with the reference method on VRBL medium incubated at 30°C and 37°C: one produced characteristic colonies on VRBL and the other is not detectable. Other *Hafnia alvei* were tested and detected with a characteristic appearance.
- The study of 20 non-coliform strains revealed negative reactions (no colonies or non-characteristic colonies) with 17 strains and positive reactions (characteristic appearance) with the 3 following strains: one *Shigella sonnei* strain and 2 *Salmonella arizonae* strains (lactose +). These three strains tested with the reference method, on VRBL medium incubated at 30°C and 37°C also produced characteristic colonies.

## **PRACTICABILITY**

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

- **Positive** and **negative** results are obtained in 24 hours with both methods (alternative and reference).

- The only differences between the RAPID'E. *Coli* 2 method and the reference method consist in the medium used and the color of the characteristic colonies.
- RAPID'E.*coli* 2 medium is used in a single layer, except for products with a very high concentration of interfering flora, and makes it possible to distinguish between *E. coli* and other coliforms.

### INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2004 with 16 participating laboratories. The analysis were carried out on samples of pasteurized milk artificially contaminated, in equivalent proportions, with an *Enterobacter cloacae* strain isolated from powdered milk and an *E. coli* strain isolated from a pastry, at the 4 following levels (in cells / ml):

- level 0
- level 1: 50 - 500
- level 2: 500 - 5000
- level 3: 5000 - 50,000

The laboratories tested, using each of the **two methods, two replicates per contamination level**. The reference method was incubated at 30°C.

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
50	40	0.20	0.31	0.19	0.22	0.069
500	40	0.16	0.31	0.16	0.30	0.043
5000	40	0.19	0.38	0.20	0.26	0.025

\* 5 laboratories did not conduct the analysis.

\* one laboratory conducted the analysis 48 hours after receipt, i.e. 72 hours after shipment and its results were not taken into account.

### Conclusion

The collaborative study demonstrates that the results obtained with the alternative method are comparable to the results obtained with the reference 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](http://www.afnor-validation.com)