



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: BRD- 07/7- 12/04

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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 β -GLUCURONIDASE-POSITIVE E.COLI 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

- Some β -D-glucuronidase-negative *E.coli* strains exist, e.g. *E.coli* O157
- Some *Salmonella* serovars and some *Shigella* species contain the β -D-glucuronidase-positive enzyme (less than 1.5% of strains)

REFERENCE METHOD

Standard EN ISO 16649-2, July 2001, Horizontal method for the enumeration of B-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-B-D-glucuronide (classification index V08-031-2).

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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
Meat products	Ground beef/ <i>E.coli</i> source pork kidneys	$y = 0.061 + 1.003 x$
Dairy products	Raw milk/ <i>E.coli</i> source raw milk	$y = -0.002 + 0.990 x$
Vegetable products	Red cabbage/ <i>E.coli</i> source red cabbage	$y = 0.037 + 0.990 x$
Seafood products	Fish fillet/ <i>E.coli</i> source flat sausage	$y = -0.188 + 1.096 x$
Cakes and pastries	Confectioner's custard/ <i>E.coli</i> source vanilla custard	$y = 0.202 + 0.951 x$

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

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

Accuracy study:

Tests were performed in 2004. The statistical interpretation was conducted on 50 results, including 45 naturally contaminated samples and 5 artificially contaminated samples, belonging to the following major food categories:

Meat products, dairy products, vegetable products, seafood products, cakes and pastries.

During the tests, the alternative method was unable to produce a result for 4 samples, unlike the reference method, due to the invasion of the agars by a high level of interfering flora

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	1.0 to 3.1
Dairy products	0.5 to 4.3
Vegetable products	1.0 to 3.8
Seafood products	2.0 to 5.5
Cakes and pastries	1.5 to 4.5

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

$$y = 0.100 + 0.968 x$$

$$R^2 = 0.994$$

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
r = 0.23

Reference method
r = 0.22

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

$$D = 0.012 \log \text{ CFU/g}$$

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

- 30 β -glucuronidase-positive *E. coli* strains were detected out of 30 tested.
- The study of 54 non-*E. coli* strains revealed negative reactions for 51 strains (no colonies or non-characteristic colonies) 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 also produced characteristic (blue) colonies on TBX- medium.

PRACTICABILITY

Use of alternative method only

- **Positive** and **negative** results are obtained in 18 hours to 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 analyses were carried out on samples of pasteurized milk artificially contaminated with a β -glucuronidase-positive *E.coli* strain isolated from a pastry at the 4 following levels (in cells / ml):

- level 0
- level 1: 10 - 100
- level 2: 100 - 1000
- level 3: 1000 - 10,000

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
50	40	0.26	0.48	0.18	0.22	0.083
500	40	0.15	0.37	0.12	0.25	0.073
5000	40	0.28	0.36	0.09	0.15	0.043

* 4 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