



**Alternative methods for agribusiness
Analytical performances certified**

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

Certificate No.: BIO 12/5 – 01/99

Validation date: 19.01.1999
Renewal date*: 06.02.2003
14.12.2006
End of validity: 19.01.2011

* EN ISO 16140 protocol was used in 2006 for the second renewal

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 qualitative analysis method:

COLI ID - medium (agar)

Selective chromogenic medium for the detection and enumeration of coliforms and β -glucuronidase-positive *E. coli* from food samples

➤ **Validated for enumeration of β -glucuronidase-positive *E. coli* at 44°C**

Protocol reference: 08142 H and I

SCOPE

All human food products

RESTRICTIONS OF USE

None

REFERENCE METHOD

ISO 16649-2: Horizontal method for enumeration of β -glucuronidase-positive *Escherichia coli* (July 2001)

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

**Deputy General Manager
Jacques BESLIN**

AFAQ AFNOR Certification

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PRINCIPLE OF THE METHOD

Coli ID medium is a chromogenic medium designed for the enumeration of coliforms and β -glucuronidase-positive *E. coli*. This medium contains two chromogenic substrates. Coliforms other than *E. coli* give blue colonies due to demonstration of β -galactosidase whereas *E. coli* colonies are pink due to the demonstration of β -glucuronidase.

NOTE

Compared to the previous validation (renewal) conducted in 2003, the protocol described in standard EN ISO 16140 was implemented.

Consequently:

- The preliminary study was repeated for linearity.
- The data obtained in 2002 were reanalyzed according to standard EN ISO 16140 for the study of accuracy.
- The specificity study was completed and the practicability study was retained.
- The inter-laboratory study was redone.

Relative LINEARITY and ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2006 on 5 food product/strain combinations within 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 contaminations 50 to 100; 100 to 500; 500 to 1,000; 1,000 to 5,000 and 5,000 to 10,000 CFU/g.

The following results were obtained:

Food category	Food matrix/strain combination	Regression line
Meat products	Minced beef / <i>E. coli</i>	$Y = 1.060 X - 0.16$
Dairy products	Milk / <i>E. coli</i>	$X = 0.993 Y + 0.014$
Seafoods	Fish fillet / <i>E. coli</i>	$Y = 0.894 X + 0.301$
Vegetables and miscellaneous	Peas / <i>E. coli</i>	$Y = 1.020 X + 0.026$
Egg products	Raw egg product/ <i>E. coli</i>	$Y = 1.127 X - 0.332$

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

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

Study of accuracy:

Tests were carried out in 2002. Statistical analysis was conducted on 121 interpretable results all of naturally contaminated samples belonging to the following main categories of foods:

Meat products, dairy products, seafoods, vegetable and miscellaneous products and egg products

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

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

Food category	Contamination scale (log cfu/g)
Meat products	0.70 to 4.36
Dairy products	0.00 to 4.52
Seafood products	0.70 to 3.69
Vegetables and miscellaneous	1.00 to 3.60
Egg products	0.70 to 4.30

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

$$\text{Equation of line: } Y = 0.97 X + 0.06$$

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 limit of repeatability (in log) obtained for the alternative method was 0.205.

The limit of repeatability (in log) obtained for the reference method was 0.088.

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

p = 0.06 for the median value.

or D= -0.02 for the average of individual biases.

Conclusion for linearity and relative accuracy:

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

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY) – 1998 and 2006 studies

Implementation of alternative method only

- 27 *E. coli* strains were detected out of the 30 tested. The 3 unrecognized strains gave non-characteristic blue colonies on Coli ID: one was a β -glucuronidase negative *E. coli* O157:H7 strain and another gave a β -glucuronidase negative reaction with an identification test strip.
- Out of 25 non *E. coli* strains tested: all the strains either presented non-characteristic colonies, or showed no growth.

PRACTICABILITY

Implementation of alternative method only

- **Response time:**

~~The Coli ID method makes it possible to obtain a result in 24h like the reference method.~~

The use of a single plate by dilution was validated.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 14 participating laboratories. The analyses were carried out on samples of partially skimmed pasteurized milk, artificially contaminated with a strain of *E. coli* serotype 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 contamination level.

The following results were obtained:

Level of Contamination	Number of laboratories giving exploitable results*	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	14	0.223	0.216	0.214	0.305	0.076
Level 2	14	0.200	0.240	0.098	0.266	0.051
Level 3	14	0.235	0.338	0.167	0.319	0.051

Conclusion

The inter-laboratory study shows that the results obtained with the alternative method are similar to those obtained with the reference method.

Please send any queries concerning the performance of the validated method to AFAQ AFNOR Certification.

On request, AFAQ AFNOR Certification will send you a summary document (in French) on the preliminary and collaborative studies.