



**Alternative methods for agribusiness
Analytical performances certified**

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

N° attestation : BIO 12/19 – 12/06

Validation date:	14.12.2006
Renewal date:	02.12.2010
End of validity:	14.12.2014

The company **BioMérieux SA**
Chemin de l'Orme
69280 Marcy L'Etoile (France)

is hereby authorized to refer to this **NF VALIDATION certificate** for the following alternative qualitative analysis method:

chromID™ Coli 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 37°C**

Protocol reference: 08142 J

SCOPE

All human food products

RESTRICTIONS OF USE

None

REFERENCE METHOD

ISO 16649-2 (2001): Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* - Part 2: colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl β -D-glucuronate

**Deputy General Manager
Jacques BESLIN**

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

PRINCIPLE OF THE METHOD

ChromID™ Coli agar (Coli ID) is a chromogenic medium used for the enumeration of coliforms and β -glucuronidase-positive *E. coli*. This medium contains two chromogenic substrates. Coliforms other than *E. coli* show up as blue colonies due to demonstration of β -galactosidase whereas *E. coli* colonies are pink due to the demonstration of β -glucuronidase.

NOTE (History of validation)

1) The present certificate concerns the use of the COLI ID method for the enumeration of *E. coli* after incubation at 37°C. The whole validation study was performed in 2006, except for the two following criteria taken from the study conducted in 1998 and 2002 during the first validation of the method (with incubation at 44°C):

- Specificity: existing data completed in 2006
- Practicability: use of previous data

2) For the renewal study of validation of December 2010, 2006 previous data of interlaboratory study were re-investigated in accordance with the draft amendment 1 to EN ISO 16140 protocol (version prA1 :2009). New results are detailed in this certificate. The chromID™ Coli agar method and the reference method remain unchanged.

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 artificial levels of contamination: 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>	$Y = 0.987 X + 0.006$
Dairy products	Milk / <i>E. coli</i>	$X = 0.990 Y + 0.025$
Seafoods	Fish fillet / <i>E. coli</i>	$Y = 0.914 X + 0.304$
Vegetables and miscellaneous	Peas / <i>E. coli</i>	$Y = 0.903 X + 0.342$
Egg products	Raw egg product/ <i>E. coli</i>	$X = 0.845 Y + 0.470$

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

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

Study of accuracy:

Tests were performed in 2006. Statistical analysis was conducted on 65 interpretable results from 49 naturally contaminated and 16 artificially 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	1.00 to 6.08
Dairy products	1.36 to 3.99
Seafoods	2.60 to 5.71
Vegetables and miscellaneous foods	1.00 to 5.95
Egg products	1.48 to 4.60

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

$$\text{Equation of line: } Y = 1.096 X + 0.099$$

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 interlaboratory 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 0.322.

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

D = - 0.04 for the median value.

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 ChromID™ Coli agar: one was a β-glucuronidase negative *E. coli* O157:H7 strain and another gave a β-glucuronidase negative reaction with an identification test strip. These β-glucuronidase-negative strains also present non-characteristic colonies on TBX agar using the reference method.
- Out of 23 non *E. coli* strains tested:
 - One strain of *Plesiomonas shigelloides* gave pink colonies.
 - The other strains either presented uncharacteristic colonies, or showed no growth

PRACTICABILITY

Implementation of alternative method only

- **Response time:**

The ChromID™ Coli agar method makes it possible to obtain a result in 24h like the reference method. The use of a single dish by dilution was validated.

INTERLABORATORY STUDY

The interlaboratory 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 results calculated in accordance with the draft amendment 1 to EN ISO 16140 standard (version prA1:2009) were the following:

Contamination level	Number of samples taken into account*	Reference method		Alternative method		Bias
		Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	
Level 1	14	0.0943	0.0943	0.0481	0.1014	0.086
Level 2	14	0.0638	0.0790	0.0832	0.0832	0.036
Level 3	14	0.0666	0.1038	0.0753	0.0988	0.092

NB: Limit of repeatability $r = 2.8 S_r$, with S_r : repeatability standard deviation
 Limit of reproducibility $R = 2.8 S_R$, with S_R : reproducibility standard deviation

Conclusion

The inter-laboratory study shows that the results obtained with the alternative method are similar to those obtained with the reference method for levels 2 and 3.

For level 1, the alternative method has a better repeatability and a limit of reproducibility less good compared to the reference method.

The biases are non-significant.

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