



Alternative methods for agribusiness  
Analytical performances certified

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

Certificate No.: AES 10/07 - 01/08

Validation date : 17.01.2008  
End of validity : 17.01.2012

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

**REBECCA™ + EB**

For enumeration of *Enterobacteriaceae*

Protocol reference: 620020: 17/01/08 -A

**SCOPE**

All products for human and animal consumption

**RESTRICTIONS OF USE**

None

**REFERENCE METHOD**

Standard NF EN ISO 21528-2 (2004): Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony counting method

A handwritten signature in black ink, appearing to read "JBESLIN".

Deputy General Manager  
Jacques BESLIN

**AFNOR Certification**

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## PRINCIPLE OF THE METHOD

REBECCA™ is a chromogenic medium for direct enumeration without confirmation in products for human and animal consumption of positive *E.coli*  $\beta$ -glucuronidase and of *Enterobacteriaceae*

## NOTE

The validation study, being the object of this certificate, was carried out with two types of seeding: seeding in mass and seeding on the surface, applied to the medium REBECCA™ + EB (supplement)

## Relative LINEARITY and ACCURACY

Comparison of the performance of the alternative method and the reference method

### Study of linearity :

Test were performed in 2007 on the 5 food products/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:

- level 1 : 30 – 300 CFU/g
- level 2 : 300 – 3 000 CFU/g
- level 3 : 3 000 – 30 000 CFU/g
- level 4 : 30 000 – 300 000 CFU/g
- level 5 : 300 000 – 3 000 000 CFU/g

The following results were obtained:

- With poured plate protocol :

Food Category	Food product.strain pair	Regression line
Minced meat	Beef/ <i>E.coli</i>	$Y = 1.016X - 0.023$
Pasteurised milk	Cantal (cheese made with raw milk / <i>E.coli</i> )	$Y = 0.999X - 0.096$
Raw fish	CIP 54.127/ <i>E.coli</i>	$Y = 1.062X - 0.399$
Frozen vegetables	Grated carrots/ <i>E.coli</i>	$Y = 0.979X + 0.094$
Cat feeding	Beef granules / <i>E.coli</i>	$Y = 1.014X - 0.297$

Y = log (N alternative method)

X = log (N reference method)

- With surface protocol :

Food Category	Food product.strain pair	Regression line
Minced meat	Beef/ <i>E.coli</i>	$Y = 1.034X - 0.334$
Pasteurised milk	Cantal (cheese made with raw milk / <i>E.coli</i> )	$Y = 1.017X - 0.135$
Raw fish	CIP 54.127/ <i>E.coli</i>	$Y = 1.007X - 0.022$
Frozen vegetables	Grated carrots/ <i>E.coli</i>	$Y = 1.006X - 0.065$
Cat feeding	Beef granules / <i>E.coli</i>	$Y = 0.956X + 0.052$

Y = log (N alternative method)

X = log (N reference method)

## Accuracy study:

Test were performed in 2007. Statistical exploitation related to 50 interpretable results coming from 38 naturally contaminated samples and 12 artificially contaminated, belonging to the following main categories major food categories.

Meat products, dairy products, seafood products, vegetable products and animal feeding products.

The samples were analyzed in duplicate by each of the two methods.

As an indication, the areas of contamination (concentration) were the following

Category of food	Contamination range (log)
Meat products	2.60-7.95
Dairy products	2.16-7.53
Seafood products	1.78-7.68
Vegetable products	1.91-8.49
Animal feeding products	1.30-5.64

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

$$\text{Equation of regression line : } Y = 0,988 X + 0,092$$

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

$$\text{Equation of regression line : } Y = 1,008 X - 0,042$$

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 accuracy criterion.

	Repeatability r alt *	Repeatability r ref. *	Bias D *
medium REBECCA <sup>IM</sup> + EB/ seeding in mass	0.244	0.210	0.038
medium REBECCA <sup>IM</sup> + EB/ seeding on surface	0.244	0.210	-0.006

*\*the results are expressed in log*

### Conclusion for relative linearity and accuracy:

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

## SELECTIVITY (INCLUSIVITY / EXCLUSIVITY)

### Implementation of alternative method only

- 30 strains of *Enterobacteriaceae* were detected out of 30 tested.
- The study of 20 strains not belonging to *Enterobacteriaceae* did not detect the presence of any cross-reaction.

## PRACTICABILITY

### Implementation of alternative method only

#### Response time :

- **Positive** results are obtained in one day using the alternative method against three days using the reference method.
- **Negative** results are obtained in one day using the alternative method against one day using the reference method.

## INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2007 with 12 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *E. coli* strain at the 4 following levels of contamination:

- 0 CFU/mL
- 10 - 100 CFU/mL
- 100 – 1 000 CFU/MI
- 1 000 – 10 000 CFU/mL

The laboratories tested, using **both methods**, **2 replicate samples** for **each level** of contamination.

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	9	0.517	0.473	0.517	0.410	0.029
Level 2	9	0.134	0.176	0.174	0.293	-0.062
Level 3	9	0.100	0.188	0.196	0.184	-0.036

\* 3 laboratories have been excluded since the analysis of results had not been shifted in time in relation to other participants.

**Conclusion**

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

(The values of repeatability of the alternative method are comparable to those of the reference method for levels 1 and 2. For level 3, the repeatability of the reference method is better than that of the alternative method.

The values of reproducibility of the alternative method are comparable with those of the method of reference for all levels of contamination.)

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

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