



Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: AES 10/06 - 01/08

Validation date : 17.01.2008
End of validity : 17.01.2012

The company AES CHEMUNEX
(head office) Rue Maryse Bastié
Ker Lann/CS 17219
35172 Bruz Cedex

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

REBECCA™ BASE or REBECCA™ + EB

For the enumeration of *E.coli* β-glucuronidase positive

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

SCOPE

All products for human and animal consumption

RESTRICTIONS OF USE

None

REFERENCE METHOD

Standard EN ISO 16649-2 (2001): horizontal method for the enumeration of *E.coli* β-D-glucuronidase positive - Part 2: technique by counting colonies at 44°C by means of 5-bromo-4-chloro-3-indolyl β-glucuronate.

**Deputy General Manager
Jacques BESLIN**

AFNOR Certification

Head office : 11, avenue Francis de Pressensé – 93571 Saint-Denis la Plaine Cedex - France
Office: 116, avenue Aristide Briand – BP 40 – 92224 Bagneux Cedex 6 – France
Tél +33 (0)1 46 11 37 00 – Fax +33 (0)1 46 11 39 40
certification@afaq.afnor.org - www.afnor-validation.com

PRINCIPLE OF THE METHOD

REBECCA™ is a chromogenic medium for direct enumeration without confirmation in products for human and animal consumption of *E.coli* β -glucuronidase positive. The enumeration of *E.coli* is done by the detection of the β -glucuronidase colouring the colonies in blue with or without a blue halo.

NOTE

The validation study, being the object of this certificate, was carried out with two types of inoculations : inoculation by poured plate and surface inoculation, each applied to two media REBECCA™ base and REBECCA™ + EB (supplement). The preliminary study was carried out with media REBECCA™ base and REBECCA™ + EB and the inter-laboratory study, with the medium REBECCA™ + EB.

Relative LINEARITY and ACCURACY

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

Study of linearity:

Tests were carried out in 2007 on the 5 food product /strain combinations and in the categories of food appearing in the table below.

The samples were analyzed in duplicate by each of the two methods, on the five following levels of artificial contamination:

- level 1: 50 - 500 CFU/g
- level 2: 100- 1,000 CFU/g
- level 3: 500 – 5,000 CFU/g
- level 4: 1,000 – 10,000 CFU/g
- level 5: 10,000 – 100,000 CFU/g

For the medium REBECCA™ base, the following results were obtained:

- with poured plate protocol:

Table of results (Cf. Table 1 of the EN ISO 16140 standard) :

Food categories	Food/strain pair	Linear regression
Minced meat	Beef/ <i>E.coli</i>	$Y = 0.969 X + 0.193$
Pasteurised milk	Cantal (cheese made with raw milk) / <i>E.coli</i>	$Y = 0.990 X + 0.063$
Raw fish	CIP 54.127/ <i>E.coli</i>	$Y = 0.948 X + 0.215$
Frozen vegetables	Grated carrots/ <i>E.coli</i>	$Y = 1.012 X - 0.018$
Cat feeding	Beef granules / <i>E.coli</i>	$Y = 1.018 X + 0.010$

- Y = log (N alternative method)

- X = log (N reference method)

- with surface protocol:

Food categories	Food/strain pair	Linear regression
Minced meat	Beef/ <i>E.coli</i>	$Y = 1.098 X - 0.182$
Pasteurised milk	Cantal (cheese made with raw milk) / <i>E.coli</i>	$Y = 0.955 X + 0.183$
Raw fish	CIP 54.127/ <i>E.coli</i>	$Y = 1.069 X - 0.169$
Frozen vegetables	Grated carrots/ <i>E.coli</i>	$Y = 0.963 X + 0.186$
Cat feeding	Beef granules/ <i>E.coli</i>	$Y = 1.010 X - 0.023$

Y = log (N alternative method)

X = log (N reference method)

For the medium REBECCA™ + EB, the following results were obtained:

- with poured plate protocol :

Food categories	Food/strain pair	Linear regression
Minced meat	Beef/ <i>E.coli</i>	$Y = 0.972 X + 0.104$
Pasteurised milk	Cantal (cheese made with raw milk) / <i>E.coli</i>	$Y = 1.008 X - 0.008$
Raw fish	CIP 54.127/ <i>E.coli</i>	$Y = 0.980 X + 0.069$
Frozen vegetables	Grated carrots/ <i>E.coli</i>	$Y = 0.914 X - 0.456$
Cat feeding	Beef granules/ <i>E.coli</i>	$Y = 0.904 X + 0.431$

Y = log (N alternative method)

X = log (N reference method)

- with surface protocol:

Category of Food	Food/strain pair	Linear regression
Minced meat	Beef/ <i>E.coli</i>	$Y = 1.139 X - 0.395$
Pasteurised milk	Cantal (cheese made with raw milk) / <i>E.coli</i>	$Y = 0.908 X + 0.373$
Raw fish	CIP 54.127/ <i>E.coli</i>	$Y = 1.088 X - 0.230$
Frozen vegetables	Grated carrots/ <i>E.coli</i>	$Y = 1.030 X - 0.091$
Cat feeding	Beef granules/ <i>E.coli</i>	$Y = 0.944 X + 0.197$

Y = log (N alternative method)

X = log (N reference method)

Study of accuracy :

Tests were carried out in 2007. Statistical exploitation related to 51 interpretable results coming from 41 samples naturally contaminated and 10 artificially contaminated, that belong to the following main categories of food :

meat products, dairy products, ocean products, vegetable products and animal feeding. The samples were analysed in duplicate by each of the two methods.

Food categories	Field of contamination (log)
Meat products	1.30 – 4.21
Dairy products	1.00 – 7.04
Seafoods	1.30 – 6.15
Frozen vegetables	1.00 – 5.87
Animal feeding	1.30 – 6.55

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

	Inoculation by poured plate	Surface inoculation
medium REBECCA™ base	$Y = 0.994 X + 0.074$	$Y = 0.990 X + 0.043$
medium REBECCA™ + EB	$Y = 1.014 X - 0.002$	$Y = 0.980 X + 0.043$

Y = log (N alternative method)

X = log (N reference method)

The repeatability for both methods and the bias between the two methods have been determined according to the method of calculation used for the collaborative study (see §6.3.5 and §6.3.6 of standard NF EN ISO 16140). These results bring additional information for the accuracy criterion

	Repeatability r_{alt}^*	Repeatability r_{alt}^*	Bias D^*
medium REBECCA ^{IM} base/ inoculation by poured plate	0.178	0.161	0.056
medium REBECCA ^{IM} base/ surface inoculation	0.238	0.161	0.010
medium REBECCA ^{IM} + EB/ inoculation by poured plate	0.227	0.161	0.044
medium REBECCA ^{IM} + EB/ surface inoculation	0.278	0.161	-0.025

* the results are expressed in log

Conclusion for relative linearity and accuracy:

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

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY) Implementation of the alternative method alone

For medium REBECCATM base and REBECCATM + EB,

- 50 strains of *E.coli* have been detected out of 50 tested.
- the study of 20 strains non-*E.coli* showed reactions with the following strain : *Shigella sonnei*

PRATICABILITY Implementation of the alternative method alone

- Time for obtaining results:
 - positive results are obtained in one day with the alternative method against one day with the reference method.
 - negative results are obtained in one day with the alternative method against one day with the reference method.

INTERLABORATORY STUDY

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

- 0 CFU/mL
- 10-100 CFU/mL
- 100- 1,000 CFU/mL
- 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 :

Contamination level	Number of laboratories results taken into account*	Reference method	Reference method	Alternative method	Alternative method	Alternative method
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Level 1	9	0.367	0.504	0.284	0.308	-0.038
Level 2	9	0.100	0.112	0.257	0.274	-0.063
Level 3	9	0.075	0.148	0.154	0.185	-0.037

*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 with those obtained with the reference method.

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

The values of reproducibility for the alternative method are comparable with those of the reference method for levels 1 and 3. For level 2, the reproducibility of the reference method is better than the one of the alternative method).

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

**You will find a summary document
(in French) on the preliminary and collaborative studies on the website
www.afnor-validation.org**