



**Alternative methods for agribusiness
Analytical performances certified**

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

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La Société
(siège social, distributeur
et site de production)

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

RAPID'E.coli O157:H7

Protocol reference: V1

SCOPE

All human food products and environmental samples

RESTRICTIONS OF USE

None

REFERENCE METHOD

EN ISO 16654 (July 2001): Microbiology of foodstuffs – Horizontal method for the detection of *Escherichia coli* O157.

A handwritten signature in black ink, appearing to read "JBESLIN", with a long horizontal stroke extending to the right.

**Deputy General Manager
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PRINCIPLE OF THE METHOD

The RAPID'E.coli O157:H7 medium is a selective medium combining both chromogenic substrates and biochemical indicators. This association enables the direct presumptive identification of RAPID'E.coli O157:H7, including atypical strains, from among interfering flora on the basis of the specific metabolic and enzymatic profiles observed.

The selectivity of the medium is increased by the addition of selective agents: novobiocin and potassium tellurite.

In the context of AFNOR VALIDATION, all positive samples at the end of the alternative method must be confirmed on the basis of isolated colonies in a chromogenic medium in accordance with the classic tests described in the methods standardised by the CEN, the ISO or the AFNOR (including the purification stage) or by the combination of two specific *Escherichia coli* O157 and H7 latex tests.

In the event of discordant results (positive by the alternative method, non-confirmed by the tests described in the CEN, ISO or AFNOR standardised methods or by the combination of two specific *E. coli* O157 and H7 latex tests), the laboratory will have to implement sufficient means to be certain of the validity of the result.

Relative ACCURACY, relative SPECIFICITY, relative SENSITIVITY Comparison of the performances of the alternative method and the reference method

In 2007, tests were carried out on 331 samples of products, 156 of which were artificially contaminated and 175 non-contaminated, belonging to the following basic categories of foodstuffs:

dairy products, meat products, vegetable products, miscellaneous and environmental samples

All the samples were analysed **individually** using the **two methods**.

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

Responses	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement A+/R+ PA = 140 ⁽¹⁾	Positive deviation A+/R- PD = 9 ⁽¹⁾
Alternative method negative (A-)	Negative deviation A-/R+ ND = 7 ⁽²⁾	Negative agreement A-/R- NA = 175 ⁽³⁾

(1) Confirmed positives

(2) of which 2 samples which were presumed positive have not been confirmed

(3) of which 3 samples which were presumed positive, displaying characteristic midnight blue colonies without a halo, were non-confirmed by the performance of latex tests. These isolates were identified as belonging to the *Escherichia fergusonii* species. 18 other samples also underwent confirmation tests. However, the colonies coloured blue to blue-green do not appear to be characteristic; latex tests, all negative, were performed with a view to guaranteeing the exclusivity of the medium.

The percentages obtained, compared to the reference method, are as follows:

- Relative accuracy: **AC = 95.2**
- Relative specificity: **SP = 95.1**

Note: a **relative specificity** of below 100 % results from a number of confirmed supplementary positives and not false positives.

- Relative sensitivity: **SE = 95.2**

Sensitivity was also recalculated taking into account all confirmed positives (including supplementary positives by alternative method):

$$\text{Alternative method:} \\ (PA + PD) / (PA + PD + ND) = 95.5$$

$$\text{Reference method:} \\ (PA + ND) / (PA + PD + ND) = 94.2$$

Conclusion

Analysis of discordants (in accordance with Appendix F of EN ISO 16140)

PD = 9, ND = 7, thus Y = PD + ND = 16; $6 \leq Y \leq 22$ m = 7, M = 3 therefore m > M

Conclusion

The two methods do not differ in statistical terms

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

In 2007, tests were carried out on five product combinations of the foodstuffs/strains described in the table below.

These products represent the following categories of foodstuffs: dairy products, meat products, vegetable products, miscellaneous and environmental samples.

The products were analysed **6 times**, using the **2 methods**, at **4 levels** of contamination.

The results obtained are the following:

Matrix	Strain	Relative detection level LOD ₅₀ (3) With confidence interval (CFU/25 g or 25 ml)	
		Alternative method	Reference method
Potted mince	<i>E. coli</i> O157:H7	0.9 [0.6 – 1.2]	0.6 [0.4 – 0.8]
Raw milk	<i>E. coli</i> O157:H7	0.8 [0.5 – 1.3]	0.7 [0.5 – 1.0]
Farmhouse cider	<i>E. coli</i> O157:H7	0.1 [0.1 – 0.2]	0.3 [0.1 – 0.9]
Mixed salad	<i>E. coli</i> O157:H7	1.2 [0.7 – 2.2]	0.5 [0.2 – 1.3]
Process water	<i>E. coli</i> O157:H7	0.7 [0.2 – 2.2]	0.4 [0.1 – 1.7]

(3) LOD₅₀: estimation of level of contamination enabling positive detection by alternative method in 50% of cases.

"Hitchins A. Proposed Use of a 50% Limit of Detection Value in Defining Uncertainty Limits in the Validation of presence-Absence Microbial Detection Methods, Draft 10th December, 2003"

Conclusion

The detection level for the alternative method is located between 0.1 and 2.2 UFC/25 g.

The detection level for the reference method is located between 0.1 and 1.7 UFC/25 g.

INCLUSIVITY/EXCLUSIVITY

Implementation of alternative method only

- 50 strains of *E. coli* O157:H7 were detected out of 50 tested.
- Out of 36 non-*E. coli* O157:H7 strains tested: two *E. coli* strains of serotypes O92:H33 and O55:H6 displayed characteristic colonies without halos. They both had negative latex tests. Two strains of *E. coli* O157 positive and H negative were tested: they display non-characteristic colonies of a grey-green colour.

PRACTICABILITY

Implementation of alternative method only

- **Response time:**
 - **Positive** results are obtained in three days with the alternative method compared to three to four days with the reference method.
 - **Negative** results are obtained in two days with the alternative method compared to one to two days with the reference method.
 - In the case of results which are presumed positive with the alternative method, but which become negative following confirmation, the negative results are obtained in three days.

INTERLABORATORY STUDY

The interlaboratory study was performed in 2007 and 21 laboratories collaborated. Analyses were carried out on samples of pasteurised milk, artificially contaminated with a strain of *E. coli* O157:H7 ATCC 700728 at the following 3 levels:

- 0 UFC/25 ml
- 1 – 10 UFC/25 ml
- 5 – 50UFC/25 ml

The laboratories tested, using **both methods, 8 replicate samples for each level** of contamination, giving a total of 24 analyses for the participating laboratories as a whole.

Results :

Contamination levels	Total n° of samples	N° of samples analysed*	N° of results exploited**	N° of negative results		N° of positive results	
				REF	ALT	REF	ALT
0	168	144	96	92***	93***	3	2
1	168	144	96	0	0	96	96
2	168	144	96	0	0	96	96

* Three laboratories did not perform the analyses and one laboratory started the analyses on Day +2

** In accordance with the AFNOR technical office, five laboratories were excluded as they obtained results which conveyed intercontaminations.

*** One container was broken in transit

Calculations

- Relative accuracy = 99.7%
- Specificity = 98.9%
- Sensitivity = 100%

Note: **relative specificity** below 100% results from a number of confirmed supplementary positives and not from false positives.

Interpretation

Results of the collaborative study are comparable to those obtained during the preliminary study.

Sensitivity was also recalculated taking into account all confirmed positive results (this includes supplementary positives with alternative method):

Alternative method:	Reference method:
$(PA + PD) / (PA + PD + ND) = 99.5\%$	$(PA + ND) / (PA + PD + ND) = 100\%$

Accordance, concordance and concordance odds ratio:

Accordance: percentage chance of finding the same result from two identical test portions analysed in the same laboratory, under repeatability conditions. The accordance is the average (mean) of the probabilities that two replicates give the same result for each laboratory.

Concordance: percentage chance of finding the same result for two identical samples analysed in two different laboratories (reproducibility conditions). The concordance is the percentage of all pairings of replicates giving the same result.

Concordance odds ratio (COR): defined by the following formula:
 $COR = \text{accordance} \times (100 - \text{concordance}) / \text{concordance} \times (100 - \text{accordance})$

The following table indicates values for the **alternative method**:

Contamination level	Accordance	Concordance	COR
L0	94%	93%	1.0
L1	100%	100%	1.0
L2	100%	100%	1.0

The following table indicates values for the **reference method**:

Contamination level	Accordance	Concordance	COR
L0	93%	92%	1.0
L1	100%	100%	1.0
L2	100%	100%	1.0

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is equivalent to that of the reference method.

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

You may download a summary document on the preliminary and inter-laboratory studies on www.afnor-validation.com