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**Microbiology of food and animal
feeding stuffs — Horizontal method
for the detection of *Escherichia coli*
O157**

**AMENDMENT 1: Annex B: Result of
interlaboratory studies**

*Microbiologie des aliments — Méthode horizontale pour la recherche
des Escherichia coli O157*

AMENDEMENT 1: Annexe B: Résultat des études interlaboratoires

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This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157

AMENDMENT 1: Annex B: Result of interlaboratory studies

Page 8, 9.1

Replace “NOTE” with “NOTE 1”.

Page 8, 9.1

Add the following at the end of the subclause.

This document has been validated for test portions of a specific weight or volume for each food matrix. A smaller test portion may be used, without the need for additional validation/verification, providing that the same ratio between (pre-) enrichment broth and test portion is maintained. A larger test portion than that initially validated may be used, if a validation/verification study has shown that there are no adverse effects on the detection of *E. coli* O157.

NOTE 2 Validation can be conducted in accordance with the appropriate documents of ISO 16140 (all parts). Verification for pooling samples can be conducted in accordance with the protocol described in ISO 6887-1:2017, Annex D (verification protocol for pooling samples for qualitative tests).

Page 11, after Clause 11

Add the following as Clause 12 and renumber the previous Clause 12 “Test report” as Clause 13 “Test report”.

12 Performance characteristics of the method

12.1 Interlaboratory study

The performance characteristics of the method were determined in interlaboratory studies to determine the specificity, sensitivity and, when possible, the LOD₅₀ of the method. The data are summarized in Annex B. The values derived from the interlaboratory studies may not be applicable to food types other than those given in Annex B.

12.2 Sensitivity

The sensitivity is the ability of the method to detect the analyte. It is defined as the number of samples found positive divided by the number of samples tested at a given level of contamination. The results are thus dependent on the level of contamination of the sample.

12.3 Specificity

The specificity is the ability of the method to correctly identify the absence of the analyte in negative samples. It is defined as the number of samples found negative divided by the number of blank samples tested.

12.4 LOD₅₀

The LOD₅₀ (level of detection) is the concentration (cfu/sample) for which the probability of detection is 50 %.

The LOD₅₀ has been calculated in the CEN Mandate studies (see Annex B) for the matrix sprouts only as the other study, on milk, returned no negative results for both the levels of contamination.

The LOD₅₀ was 8,4 cfu/g (6,5 cfu/g to 10,9 cfu/g confidence limit, 95 % confidence interval).

Page 13, after Annex A

Add the following as Annex B.

Annex B (informative)

Results of interlaboratory studies

B.1 Results of interlaboratory studies of an NMKL study

An international interlaboratory study was organized in 1999 by the Nordic Committee on Food Analysis on the following food matrices: minced meat, raw milk and lettuce (see Reference [1]) on method assessed as equivalent to the method ISO 16654:2001.

The values of the performance characteristics derived from this interlaboratory study are shown per type of sample in [Tables B.1](#) to [B.3](#).

Table B.1 — Results of data analysis obtained with 25 g minced meat samples

Performance characteristic	Minced meat (blank)	Minced meat (low level, 17 cfu/sample)	Minced meat (high level, 80 cfu/sample)
Year of interlaboratory test	1999	1999	1999
Number of laboratories having returned results	14	14	14
Number of samples per laboratory	2	2	2
Number of excluded laboratories	0	0	0
Number of laboratories retained after the exclusion	14	14	14
Number of accepted samples	28	28	28
Sensitivity in %	–	100	100
Specificity in %	92,8	–	–

Table B.2 — Results of data analysis obtained with 25 ml raw milk samples

Performance characteristic	Raw milk (blank)	Raw milk (low level, 17 cfu/sample)	Raw milk (high level, 78 cfu/sample)
Year of interlaboratory test	1999	1999	1999
Number of laboratories having returned results	14	14	14
Number of samples per laboratory	2	2	2
Number of excluded laboratories	0	0	0
Number of laboratories retained after the exclusion	14	14	14
Number of accepted samples	28	28	28
Sensitivity in %	–	92,8	100
Specificity in %	85,7	–	–

Table B.3 — Results of data analysis obtained with 25 g lettuce samples

Performance characteristic	Lettuce (blank)	Lettuce (low level, 10 cfu/sample)	Lettuce (high level, 33 cfu/sample)
Year of interlaboratory test	1999	1999	1999
Number of laboratories having returned results	14	14	14
Number of samples per laboratory	1	2	2
Number of excluded laboratories	0	0	0
Number of laboratories retained after the exclusion	14	14	14
Number of accepted samples	14	28	28
Sensitivity in %	–	100	100
Specificity in %	85,7	–	–

B.2 Results of interlaboratory studies of a CEN Mandate study

B.2.1 Study on milk

A second international interlaboratory study was run in 2012 in the framework of the CEN Mandate M/381.

In the preliminary steps of the process, the method ISO 16654:2001 was evaluated as being equivalent to the NMKL method N. 164 (See resolution 297 taken at the ISO/TC 34/SC 9 meeting in Prague in June 2006 for reference). Given the equivalence assessment between the two methods, at the 14th meeting of CEN/TC 275/WG 6 (23 to 28 April 2007, Cairo, Egypt) it was agreed to perform the validation study of the method ISO 16654:2001 in a reduced form, including only one epidemiologically relevant food matrix (milk).

National reference laboratories (NRLs) for *E. coli*, and laboratories involved in the official controls of foodstuffs of EU Member States were selected based on whether they were accredited according to ISO 17025, had the method ISO 16654:2001 accredited and had expertise in IMS-based isolation of food-borne bacteria.

In total, 15 laboratories participated in the inter-laboratory study, which was based on the analysis, with the method ISO 16654:2001, of raw milk samples artificially contaminated with *E. coli* O157 at three different levels of contamination: blank (0 cfu/ml), low (25 cfu/ml, combined uncertainty $U_c = 7$ cfu/ml) and high (140 cfu/ml, $U_c = 38$ cfu/ml). The samples contained naturally present background microflora, and eight blind replicates of each level of contamination were sent to each laboratory, for a total of 24 samples examined. The samples contained glycerol and were sent to the laboratories as frozen specimens (-70 °C in dry ice).

The values of the performance characteristics derived from this collaborative test is shown in [Table B.4](#).

Table B.4 — Results of data analysis obtained with 10 ml milk samples

Performance characteristic	Milk (blank)	Milk (low level, 250 cfu/sample)	Milk (high level, 1 400 cfu/sample)
Year of interlaboratory test	2012	2012	2012
Number of laboratories having returned results	15	15	15
Number of samples per laboratory	8	8	8
Number of excluded laboratories	0	0	0
Number of laboratories retained after the exclusion	15	15	15
Number of accepted samples	120	120	120
Sensitivity in %	–	100	100
Specificity in %	94,4	–	–

B.2.2 Study on sprouts

A third international interlaboratory study was organized and run in 2013 involving laboratories enrolled according to the same principles used for the first CEN mandate study on milk.

Fourteen laboratories received a total of 24 samples consisting of 8 blind replicates of sprouts, purchased at retail, spiked with 3 different concentrations of *E. coli* O157: 0 cfu/g, 10 cfu/g and 100 cfu/g. The extended uncertainty of measurement associated with the inoculum was 0,27 log cfu/ml (calculated according to the ISO/TS 19036). The sprout samples each contained a mixture of alfalfa (90 %) and watercress (10 %), with an endogenous microflora estimated to be 10⁵ cfu/g to 10⁷ cfu/g and have been sent refrigerated to the participating laboratories.

The values of the performance characteristics derived from this collaborative test are shown in [Table B.5](#).

Table B.5 — Results of data analysis obtained with 25 g sprout samples

Performance characteristic	Sprouts (blank)	Sprouts (low level, 250 cfu/sample)	Sprouts (high level, 2 500 cfu/sample)
Year of interlaboratory test	2014	2014	2014
Number of laboratories having returned results	14	14	14
Number of samples per laboratory	8	8	8
Number of excluded laboratories	0	0	0
Number of laboratories retained after the exclusion	14	14	14
Number of accepted samples	112	112	112
Sensitivity in %	–	75,9	96,4
Specificity in %	99,1	–	–

Bibliography

Add the following reference.

- [7] NMKL N. 164:1999, *Escherichia coli* O157. Detection in food and feeding stuffs