AFNOR Validation

Alternative methods for agribusiness
Analytical performances certified

VALIDATION CERTIFICATE ACCORDING TO THE NF V 03-100 STANDARD

Certificate N° : TRA 02/6 - 11/95

Validation date :	**21.11.95**
First renewal date :	**07.02.2000**
Second renewal date :	**11.12.2003**
End of validity :	**21.11.2007**

The Company (Head office, distributor & production site)
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is authorized to use this AFNOR validation certificate as a reference document for the following alternative analysis method :

TRANSIA® PLATE Listeria

Protocol reference : NOT COM 020J 01/04 (Art. Nr LI 0691: 1 microplate)
NOT COM 420E 01/04 (Art. Nr LI 0685: 10 microplates)

METHOD PRINCIPLE

ELISA test based on a Sandwich-type reaction and performed after two steps: a selective enrichment followed by a heat inactivation allowing the release of the *Listeria* antigens. The colorimetric reaction developed then in a well of a microtitreplate is assessed with a microplate reader.

In the context of AFNOR Validation, all samples identified as positive by the TRANSIA PLATE Listeria method must be confirmed by one of the following means:

- According to classical tests described in methods standardized by CEN, ISO or AFNOR (including a purification step), starting from the Fraser broth.
- With a Microbact 12L gallery (on 1 to 4 isolated suspicious colonies tested without prior purification) after a streaking of the non-heated Fraser broth on a chromogenic agar according to Ottaviani & Agosti
- Or by implementing any other AFNOR validated method based on a principle different from the TRANSIA PLATE Listeria method, respecting specifications in the test instructions.

SCOPE : All foods for human consumption.

RESTRICTIONS OF USE : None

REFERENCE METHODS

- For the renewal study (2003)
  EN ISO 11290-1 (February 1997): Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method.

- For the initial validation study (1994)
  French standard V 08-055 (December 1993): Food microbiology - Detection of *Listeria monocytogenes* - Routine method.

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NOTE

In 2003, the validation was renewed with a modified protocol:
- Incubation times were changed to 20 to 26 hours for the first incubation and 22 to 26 hours for the second;
- Incubation temperature of the subculture in Fraser broth was changed from 26 °C to 30 °C.

PRACTICABILITY (study performed in 2003)

- Positive results are obtained in 3 to 4 days using the TRANSIA PLATE Listeria method, compared with 3 to 6 days using the reference method.

- Negative results are obtained in 2 days using the TRANSIA PLATE Listeria method, compared with 2 to 5 days using the reference method.

- Positive results with the TRANSIA PLATE Listeria method followed by a negative confirmation with the reference method are obtained in 3 to 4 days.

SPECIFICITY (study performed in 1994)

- 107 *Listeria* strains were detected out of 108 tested strains. The non-detected strain was a non-motile *L. monocytogenes* 1/2.

- The study of 50 strains not belonging to the *Listeria* genus did not detect any presence of cross-reactions

INTRINSIC DETECTION LIMIT (study performed in 2003)

Number of *Listeria* required in order to obtain a positive result with the TRANSIA PLATE Listeria method: 1.3 10^4 to 3.8 10^4 cells/mL (this result was obtained on 4 pure strains of *Listeria*: *L. monocytogenes* 4b, *L. monocytogenes* 1/2 a, *L. innocua* and *L. welshimeri*.).

DETECTION LIMIT (study performed in 2003)

Assays were performed in 2003 using 4 different types of food (raw milk, traditional potted pork meat, raw vegetables and smoked fish fillet), each artificially contaminated with 4 strains of *Listeria*, at 5 different contamination levels (0, 1 to 10 CFU/25g, 2 to 20 CFU/25g, 5 to 50 CFU/25g, 10 to 100 CFU/25g).

The following results were obtained:

Level of contamination in CFU/25 g	Actual level in CFU/25g	Alternative method % of detected samples	Reference method % of detected samples
0	0	0	0
1 to 10	4 to 7	100	100
2 to 20	8 to 15	100	100
5 to 50	20 to 37	100	100
10 to 100	39 to 73	100	100

Conclusion

Concordance between the reference method and the TRANSIA PLATE Listeria method is 100 %. The lowest contamination levels which were tested (between 4 and 7 CFU/25g) were detected.

ACCURACY (study performed in 2003)

Comparison of performance between the alternative method and the reference method

Assays were performed in 2003, using 218 samples (meat products, vegetables, dairy products and seafood products), of which 86 were naturally contaminated, 36 artificially contaminated and 93 naturally uncontaminated.

All samples were analyzed in single by the alternative method and the reference method.

Results were as follows:

- False negatives : 3
- False negative reference method : 1 (= additional positive)
- Concordant results: 214

Conclusion

Concordance between the TRANSIA PLATE Listeria method and the reference technique is 98.2 %.

PRECISION (study performed in 2003)

Precision data were determined during an assay performed in 2003, involving 14 laboratories. Analyses were performed using pasteurized milk artificially contaminated with a *Listeria monocytogenes* 1/2b strain at 4 different levels: 0, 1-10 cells/mL, 5-50 cells/mL, and 10-100 cells/mL.

Laboratories tested 2 samples for each level of contamination.

Results per contamination level were as follows:

Level (cells/25g)	Total number of samples	Number of sample analyzed (*)	Number of negative results	Number of positive results	Number of unprocessed samples (**)
0	26	24	22	0	2
1 - 10	26	24	0	22	2
5 - 50	26	24	0	22	2
10 - 100	26	24	0	22	2

(*) 2 laboratories did not perform the analyses because of a late delivery of the samples.
(**) the results of 1 laboratory were not taken into account, due to a too high temperature of the samples upon delivery.

Conclusion

Agreement is 100 % compared with expected results.
The method is reliable.

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

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