



**VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYSIS METHOD
ACCORDING TO THE NF V 03-100 STANDARD**

Certificate N° : GEN – 25/02 – 07/04

Validation date : 02.07.2004
End of validity : 02.07.2008

The French version of this certificate is to be used as a reference

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is authorized to use this AFNOR validation certificate as a reference document for the following alternative analysis method :

GeneDisc Cycler *Listeria monocytogenes*

Protocol reference : GDLISMO-06.04 and PELIS03/100.01

METHOD PRINCIPLE

The GeneDisc Cycler *Listeria monocytogenes* kit (GeneDisc Lis) is an automated and miniaturised system designed to detect *Listeria monocytogenes*. The detection is based on real time PCR analysis of DNA which has been first extracted with the Extraction Pack. DNA target is amplified within the GeneDisc, by the means of a fine network of microchannels which automatically transfer the purified bacterial DNA sample from each reservoir into the reaction chambers which are preloaded with the reagents necessary for detecting / quantifying required target. Results are obtained by a fluorescence measure emitted by a market probe.

As far as AFNOR validation is concerned, all positives samples issued from GeneDisc Lis method must be confirmed in the following ways :

- according to classical tests described in the standardised methods by CEN, ISO or AFNOR (including the purification step) from enrichment medium.
- spreading 0.1 ml of enrichment medium on ALOA ® medium and following instructions described in the protocol referenced above.

In case of unmatched results (positive with the alternative method and not confirmed with one of the methods above) the laboratory will have to implement sufficient means to ensure the validity of the return result.

SCOPE

All food products, except dairy products.

RESTRICTIONS OF USE : None

REFERENCE METHOD

NF EN ISO 11290-1 : microbiology of food – Horizontal method for the detection and quantification of *Listeria monocytogenes* – Part 1 : Research method (February 1997).

Deputy General Manager
Jacques BESLIN

PRACTICABILITY

- Positive results are obtained (confirmed by spreading on ALOA ® medium) in 2 days using the GeneDisc Lis, compared with 7 to 11 days using the reference method.
- Negative results are obtained in 1 day using the GeneDisc Lis, compared with 5 to 11 days using the reference method.
- Presumptive positive results using the GeneDisc Lis with a negative confirmation by the reference method are obtained in 24 hours.

SPECIFICITY

- 50 *Listeria monocytogenes* strains were detected out of 50 strains tested.
- The study of 30 strains not belonging to the genus *Listeria monocytogenes* did not detect the presence of cross-reactions.

INTRINSIC DETECTION LIMIT

The number of *Listeria monocytogenes* required to obtain a positive result with the GeneDisc Lis is contained between 40 and $5.0 \cdot 10^2$ UFC / ml.

This result was obtained on pure strains of *Listeria monocytogenes* belonging to the following serotypes : 1/2a, 1/2b, 4b, 4c.

DETECTION LIMIT

Assays were performed in 2003 using 3 food matrices (traditional rillettes, smoked salmon, mixture of carrots and celery) each artificially contaminated with 4 strains of *Listeria monocytogenes* (serotypes 1/2a, 1/2b, 4b, 4c) at 5 different contamination levels (0, 1-10, 2-20, 5-50, 10-100 UFC/25 g).

Additional assays have been made on minced beef, with the above strains, and the low levels : 0, 1-10, 2-20 UFC/25 g.

Each product was double tested by the GeneDisc Lis and the reference method.

The following results were obtained :

Level of contamination in CFU/25 g	Real level CFU/25 g	Alternative method % of samples detected	Reference method % of samples detected
0	0	0	0
1 to 10	3 to 10	100	100
2 to 20	7 to 20	100	100
5 to 50	15 to 48	100	100
10 to 100	31 to 97	100	100

Conclusion

The detection limit of the GeneDisc Lis method is between 1 and 10 CFU/25g.

ACCURACY

Comparison of performance between the alternative method and the reference method

Assays were performed in 2003, using 191 samples of which 64 naturally contaminated, 99 naturally uncontaminated and 28 artificially contaminated, belonging to the main following classes food products: meat products, fish products, vegetable and various.

Samples were single tested by both methods.

Results were as follows :

- False negative : 5
- Additional positive alternative method : 4 (in fact, confirmed positive)
- Concordant results : 182

Conclusion :

Concordance between the GeneDisc Lis and the reference method is 95.3 %.

PRECISION

Precision data were determined during a assay performed in 2004, involving 9 laboratories. Analysis were performed using minced beef samples, artificially contaminated with *Listeria monocytogenes* V8/127 strains at 4 different levels : 0, 1-10, 5-50, 10-100 UFC/25 g.

Laboratories double tested one sample for each level of contamination, and for each protocol.

Results per contamination level were as follows :

Level (UFC/mL)	Total number of samples	Number of samples analyzed	Number of processed samples	Number of negative results	Number of positive results
0	18	18	18	18	0
1 to 10	18	18	18	0	18
5 to 50	18	18	18	0	18
10 to 100	18	18	18	0	18

Conclusion

The global percentage of concordance with the expected results is 100%.
The method is reliable.

NOTE :

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.