afnor CERTIFICATION

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Ref.: SSO/JFY/NF102/Clients/Europrobe/
Avis BT_Prolongation LUMIPROBE 24 Salmonella_2017-07-04

Subject: NF VALIDATION mark

EUROPROBE
Mrs Nicole MALARRE
Le Gemellyon Nord
57 Bd Vivier Merle
F-69429 LYON Cedex 03
FRANCE

La Plaine Saint-Denis, July 4th, 2017

Dear Madam,

The NF VALIDATION certificate of the following analysis method:

LUMIPROBE 24 SALMONELLA SPECIES	**Ref. EUR 15/02-11/00**

will expire on July 4th, 2017 before that complete results of the renewal study may be examined by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102).

Following the positive agreement of the Technical Board, I declare that you can continue to refer to this certificate till November 24th, 2017.

Yours Sincerely.

Managing Director
Franck LEBEUGLE

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Ref.: SSO/JFY/NF102/Clients/Europrobe/
Avis BT_Prolongation LUMIPROBE 24 Salmonella_2017-05-19

Subject: NF VALIDATION mark

EUROPROBE
Mrs Nicole MALARRE
Le Gemellyon Nord
57 Bd Vivier Merle
F-69429 LYON Cedex 03
FRANCE

La Plaine Saint-Denis, May 19th, 2017

Dear Madam,

The NF VALIDATION certificate of the following analysis method:

LUMIPROBE 24 SALMONELLA SPECIES	**Ref. EUR 15/02-11/00**

will expire on May 29th, 2017 before that complete results of the renewal study may be examined by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102).

Following the positive agreement of the Technical Board, I declare that you can continue to refer to this certificate till July 4th, 2017.

Yours Sincerely.

Managing Director
Franck LEBEUGLE

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Ref.: SSO/JFY/NF102/Clients/Europrobe/
Avis BT_Prolongation LUMIPROBE 24 Salmonella_2016-11-22

Subject: NF VALIDATION mark

EUROPROBE
Mrs Nicole MALARRE
Le Gemellyon Nord
57 Bd Vivier Merle
F-69429 LYON Cedex 03
FRANCE

La Plaine Saint-Denis, November 22nd, 2016

Dear Madam,

The NF VALIDATION certificate of the following analysis method:

LUMIPROBE* 24 *SALMONELLA SPECIES	**Ref. EUR 15/02-11/00**

will expire on November 29th, 2016 before that complete results of the renewal study may be examined by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102).

Following the positive agreement of the Technical Board, I declare that you can continue to refer to this certificate till May 29th, 2017.

Yours Sincerely.

Managing Director
Franck LEBEUGLE

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: EUR 15/02 – 11/00

Validation date:	**29.11.2000**
Extension date:	**07.03.2002**
Renewal dates*:	**08.04.2005***
	18.05.2009
	29.11.2012
End of validity:	**29.11.2016**

**** EN ISO 16140 protocol was used in 2005 for the preliminary study and in 2008 for the interlaboratory study***

The Company (Head Office)
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Distributor
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is hereby authorized to refer to this **NF VALIDATION certificate** for the following alternative **qualitative** analysis method:

LUMIPROBE 24 SALMONELLA SPECIES

Protocol references: FTST-V4-13 (tube) and FTSP-V4-13 (microplate)

SCOPE

All human and animal food products.

RESTRICTIONS

None.

REFERENCE METHOD

EN ISO 6579 (December 2002): Microbiology of food and animal feedings stuffs. Horizontal method for the detection of *Salmonella* spp.

Managing Director
Florence MÉAUX

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CERTIFICATION DE PRODUITS ET SERVICES
ACCRÉDITATION N° 5-0030 PORTÉE DISPONIBLE SUR WWW.COFRAC.FR

PRINCIPLE OF THE METHOD

The *LUMIPROBE 24 SALMONELLA SPECIES* method is a test which associates an enrichment in specific broths and a detection step by hybridisation of nuclear probes in solid phase, rapid and specific of *Salmonella*. The rRNA of the targeted bacteria, released by lysis, is captured by an oligonucleotide coated on a support. It is then combined by hybridisation with a second oligonucleotide, labelled by a tracer; hybrids are revealed by a chemiluminescent reaction.

In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed, starting from the selective broth used for *LUMIPROBE 24 SALMONELLA SPECIES* test (RV), by classical tests described in methods standardized by CEN or ISO (including a purification step).

In the event of discordant results (presumptive positive with alternative method, non-confirmed by means of options described above) the laboratory must follow the necessary steps to ensure validity of the result obtained.

Note: The alternative method has been validated for the standard protocol (all human and animal food products).

NOTE (History of validation)

1/ In 2005, the renewal of the validation has taken in account the following changes, compared to the first validation in 2000 (egg products), extended to all products in 2002:

- New reference method EN ISO 6579 (2002) replacing EN 12824
- New validation protocol according to EN ISO 16140 standard

Some tests run in 2000 and 2002 have been kept interlaboratory study realized according to NF VALIDATION rules for study-Rev 7), and the comparison study has been completed on the following points : accuracy, relative sensitivity and specificity, relative detection level, inclusivity.

2/ In May 2009, the validation of the kit was renewed. Since the last renewal, the alternative method, as well as the reference method, was not modified. To comply with the requirements of the EN ISO 16140 standard, the inter-laboratory study was completely repeated. Also, complementary assays were performed for the dairy products food category (relative accuracy/specificity/sensitivity, relative detection level and inclusivity). All results are detailed in this certificate.

3/ In November 2012, the validation of *LUMIPROBE* 24 *SALMONELLA SPECIES* was renewed. The alternative method was not modified, and the reference method and the validation protocol remained the same. The selectivity study was completed in accordance with NF VALIDATION specific requirements. The results conformed to those expected and are presented in this certificate.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

In 2005 tests were carried out in 2005 for the 1st validation renewal, and then completed in 2008 for the 2nd validation renewal, on a total of 334 product samples, of which 51 were naturally contaminated, 110 artificially contaminated, and 173 non-contaminated, belonging to the following principal food product categories:

- Meat products,
- Seafood products and vegetables,
- Dairy products,
- Egg products
- Pet food products

All samples were analysed **in single** by the **two methods.**

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

	Reference method positive (R+)	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement A+ / R+ PA = **151** (1)	Positive agreement A+ / R- PD = **3** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **7** (2)	Negative agreement A- / R- NA = **173** (3)

(1) Confirmed positives
(2) (3) Of which none sample presumed positive by the alternative method was negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy: **97.0%**
- Relative specificity: **98.3%**
- Relative sensitivity: **95.6%**

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

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

Alternative method:

(PA + PD) / (PA + PD + ND) = 95.6%

Reference method:

(PA + ND) / (PA + PD + ND) = 98.1%

Conclusion

Analysis of discrepant results (according to appendix F of standard EN ISO 16140):

PD = 3, ND = 7; Y = PD + ND = 10 ; $6 \leq Y \leq 22$; m = 3, M = 1 ; so m > M

Conclusion

The two methods are not statistically different.

Relative DETECTION LEVEL

Comparison of performances of the alternative method and the reference method

Tests were carried out in 2005 for the 1st validation renewal, and then in 2008 for the 2nd validation renewal, on a total of 5 combinations of "food product/strain" described in the table below.

These products represent the following food products categories:

- Meat products
- Seafood products
- Vegetables and dairy products
- Egg products
- Pet food products

Products were analysed **6 times** by the **2 methods** at **4 levels** of contamination.

Results obtained are as follows:

		Relative detection level (CFU/25g or 25 ml) With confidence interval (3) LOD_{50}	
Matrix	**Strain**	**Alternative method**	**Reference method**
Study of 2005			
Whole eggs	*Salmonella* Enteritidis S38	0.6 [0.4 - 1.0]	0.8 [0.5 – 1.2]
Raw minced meat	*Salmonella* Typhimurium S15	1.6 [1.0 – 2.4]	1.3 [1.0 – 1.8]
Raw milk	*Salmonella* Dublin S59	8.2 [5.6 – 11.9]	1.9 [1.1 – 3.4]
Smoked salmon	*Salmonella* Enteritidis S63	1.0 [0.6 – 1.7]	1.0 [0.6 – 1.7]
Granules for rodents	*Salmonella* spp S65	0.6 [0.4 – 1.1]	1.0 [0.6 – 1.7]
Study of 2008			
Raw milk	*Salmonella* Dublin	0.8 [0.6 – 1.2]	0.9 [0.7 – 1.3]
Raw milk	*Salmonella* Newport	0.6 [0.5 – 0.8]	0.6 [0.4 – 0.8]

(3) **LOD_{50}**: estimation of level of contamination enabling positive detection by alternative method in 50% of cases. *FDA. 2006. Final Report and Executive Summaries from the AOAC International Presidential Task Force on Best Practices in Microbiological Methodology. Appendix K. Statistics Working Group (Tholen, D. W., D. S. Paulson, B. Jarvis, D. M. Mettler, B. Lombard, K. Newton, M. A. Mozola, and A. D. Hitchins.) Report Part 4a - LOD50.*

Conclusion

The detection level of the alternative method is between 0.4 and 11.9 CFU/25g.
The detection level of the reference method is between 0.4 and 3.4 CFU/25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- **Inclusivity:**
  - 2000 and 2002 studies: 50 strains of *Salmonella* were detected out of 50 tested.
  - 2005 complementary study: The 6 tested strains have been detected (1 Typhi, 2 Paratyphi A, 2 Paratyphi B, 1 Paratyphi C).
  - 2008 study: 14 other strains of *Salmonella* were detected out of 17 tested. 3 strains of *Salmonella* Gallinarum were not detected initially. For a contamination level between 50 to 70 CFU/225 ml, the detection threshold of *LUMIPROBE 24 Salmonella SPECIES* kit was not reached for this serovar growing slower than other serovars of *Salmonella*.
  - 2012 study (3rd validation renewal): 10 other strains of *Salmonella* (among which 3 were non-motile strains of *Salmonella* Typhimurium) were detected out of 10 tested.
- **Exclusivity:**
  - 2000 and 2002 studies:

    The study of 30 strains not belonging to the specie *Salmonella* showed cross reactions with 2 strains of *Citrobacter diversus* (*C. diversus* 140 and *C. diversus* CIP8294) and 1 strain of *Enterobacter sakazakii* 95. Those cross reactions were not found on liquid egg; the tests of 7 others strains of *E. sakazakii* gave negative results.

Two strains of *Citrobacter freundii* (23 and 175), and one strain of *Enterobacter agglomerans* (II) showed a cross reaction when testing BHI, but this was not the case using the protocol of *LUMIPROBE* method (RM+RV).

- 2012 study (3rd validation renewal): The study of 4 strains not belonging to the specie *Salmonella* did not show cross reaction.

PRACTICABILITY

Implementation of alternative method only

- **Response time:**

- **Positive** results are obtained in 4 to 6 days using the alternative method against 5 to 7 days using the reference method.
- **Negative** results are obtained in 1 day using the alternative method against 5 to 7days using the reference method.
- In the case of results positive presumptive results using the alternative method, but found negative following confirmation, these negative results are obtained in 3 to 5 days.

- **Personnel training:** One day training for an operator trained in classical tests of microbiology.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2008 with 12 participating laboratories. The analyses were carried out on samples of pasteurized milk artificially contaminated with a strain of *Salmonella* Enteritidis at the following 3 levels of contamination:

- level 0 : 0 CFU/ml
- level 1 : 3 CFU/ml
- level 2 : 30 CFU/ml

The laboratories tested, using **both methods**, **8 replicate samples** for **each level** of contamination, giving 48 analyses for each participating laboratory.

The following results were obtained:

Contamination level	Total number of samples	Number of samples analysed	Number of results processed *	Number of negative results		Number of positive results	
				REF	ALT	REF	ALT
0	96	96	88	87	88	1	0
1	96	96	88	6	12	82	76
2	96	96	88	1	1	87	87

** The results of one laboratory were not taken into account because the protocol of the alternative method was not respected.*

Calculations

- Relative accuracy = **92%**
- % specificity = **100%**
- % sensitivity = **92.6%**

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) = 96%	(PA + ND) / (PA + PD + ND) = 92%

Accordance, concordance and concordance odds ratio:

Accordance: percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions analysed in the same laboratory, under repeatability conditions (i.e. one operator using the same apparatus and same reagents within the shortest feasible time interval). 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. The concordance is the percentage of all pairings of duplicates giving the same result

Concordance odds ratio (COR): defined by the following formula:
COR= accordance x (100 - concordance) / concordance x (100 – accordance)

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

Contamination level	Accordance %	Concordance %	COR
L0	100	100	1.00
L1	78	78	1.00
L2	98	98	1.00

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

Contamination level	Accordance %	Concordance %	COR
L0	98	98	1.00
L1	90	87	1.30
L2	98	98	1.00

Conclusion

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

Please send any queries concerning the performance of the validated method to AFNOR Certification.
You may download a summary document on the preliminary and inter-laboratory studies on www.afnor-validation.com