afnor CERTIFICATION

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Ref.: SSO/AYV/NF102/Clients/ROMER LABS/
Avis BT_RapidChek Select Salmonella_2014-03-20_(R1)

Subject: NF VALIDATION mark

ROMERS LABS Inc.
Mrs. Meredith SUTZKO
130 Sandy Drive
NEWARK, DE 19713
U.S.A.

La Plaine Saint-Denis, March 26th, 2014

Dear Madam,

Following the positive agreement expressed the March 20th, 2014, by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102), I beg to inform you that the **NF VALIDATION certification is renewed** for the following alternative analysis method:

RapidChek® SELECT *Salmonella*

Validated for the detection of *Salmonella* in all human food, animal feed and production environmental samples (except primary production environment)

Certificate reference n# SDI 34/01-04/10, with end of validity 2nd-April-2018

A further letter will mention full conclusions and possible reservations made by the Technical Board. If reservations are mentioned, I ask you to take them into account without any delay.

Yours Sincerely.

Managing Director
Florence MÉAUX

AFNOR Validation

Alternative methods for agribusiness
Analytical performances certified

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

Certificate No.: SDI 34/01 – 04/10

Validation date :	**2010.04.02**
End of validity :	**2014.04.02**

The company (head office and Production site)	**SDIX** 111 Pencader Drive Newark, DE 19702 USA	**Distributor**	**SDIX Europe Ltd.** Barnes Wallis House 25 Barnes Wallis Road Segensworth East Hampshire, PO15 5TT UK

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

RapidChek® SELECT *Salmonella*

Part Numbers 7000190-7000197

Protocol reference: Part Number 3090045 - **V.4**

SCOPE

All human food, animal feed and environmental samples (except breeding samples).

RESTRICTIONS OF USE

The method does not allow the detection of *Salmonella* belonging to the group "O:18" (K for the old designation).

REFERENCE METHOD

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

Deputy General Manager
Jacques BESLIN

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PRINCIPLE OF THE METHOD

The RapidChek SELECT *Salmonella* method is an immunoassay test using a lateral flow test strip in a double antibody sandwich forma. The kit permits the presumptive detection of the *Salmonella* spp after 24 hours of enrichment.

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

- According to classical tests described in methods standardized by CEN or ISO (including a purification step), starting from RapidChek SELECT enriched secondary media and streaking onto two different selective agars such as XLD and ASAP.

In the event of discordant results (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.

Relative ACCURACY, relative SPECIFICITY and relative SENSITIVITY

Comparison of performances of the alternative method and the reference method

In 2009 tests were carried out on 380 product samples, of which 50 were naturally contaminated, 136 artificially contaminated, and 194 non-contaminated, belonging to the following principal food product categories:

Meat products, dairy products, seafood and vegetables products, egg products, feedstuffs and environmental samples.

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 = **168** (1)	Positive deviation A+ / R- PD = **6** (1)
Alternative method negative (A-)	Negative deviation A- / R+ ND = **12** (2)	Negative agreement A- / R- NA = **194** (3)

(1) Confirmed positives

(2) Of which 3 samples presumed positive by the alternative method were negative after confirmation

(3) Of which 22 samples presumed positive by the alternative method were negative after confirmation

Percentages obtained compared to the reference method are as follows:

- Relative accuracy : **AC = 95.3%**
- Relative specificity: **SP = 97.0%**

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

- Relative sensitivity: **SE = 93.3%**

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

Alternative method :	Reference method :
(PA + PD) / (PA + PD + ND) = 93.5%	(PA + ND) / (PA + PD + ND) = 96.8%

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

PD = 6, ND = 12, Y = PD + ND = 18 ; $6 \leq Y \leq 22$, m = 6, M = 4, 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 2009, on 6 combinations of food products/strains.

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**
Minced meat	S. Typhimurium	1.2 [0.9 – 1.5]	0.9 [0.7 – 1.1]
Raw milk	S. Newport	0.7 [0.5 – 0.9]	0.6 [0.4 – 0.8]
Raw fish	S. London	0.9 [0.7 – 1.2]	0.7 [0.6 – 0.8]
Egg	S. Enteritidis	0.7 [0.5 – 1.1]	0.6 [0.4 – 0.9]
Cat food	S. Infantis	1.3 [0.8 – 2.2]	0.9 [0.6 – 1.4]
Process water	S. Mbandaka	0.8 [0.6 – 1.2]	0.9 [0.6 – 1.1]

(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 relative detection level of the alternative method is comprised between 0.5 and 2.2 CFU/25g.
The relative detection level of the reference method is comprised between 0.4 and 1.4 CFU/25g.

INCLUSIVITY / EXCLUSIVITY

Implementation of alternative method only

- 43 strains of *Salmonella* were detected out of 51 tested. Five strains did not grown in RapidChek Primary medium (S. Derby, S. Gallinarum, S. Havana, S. Paratyphi C and S. Typhimurium) and others three strains (S. Arizonae, S. Cerro, S. Montevideo) gave negative results.

  Additional tests were performed on four strains of *Salmonella* of group O:18 (S. Carnac, S. Toulon and two S. Cerro). All results were negative by the alternative method, but positive with the reference method.

- The study of 30 strains non *Salmonella* did not detect the presence of any cross-reaction.

PRACTICABILITY

Implementation of alternative method only

- **Response time :**
  - **Positive** results are obtained in 3 to 4 days using the alternative method against 4 to 5 days using the reference method.
  - **Negative** results are obtained in 1 day using the alternative method against 3 days using the reference method.
  - In the case of results presumed positive using the alternative method, but rendered negative following confirmation, these negative results are obtained in 4 days.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2010 with 14 participating laboratories. The analyses were carried out on samples of pasteurized milk, artificially contaminated with a *Salmonella* Enteritidis strain at the 4 following 3 levels of contamination:

- Level 1 : 0 CFU / 25 mL
- Level 2 : 3 CFU / 25 mL
- Level 3 : 30 CFU / 25 mL

The laboratories tested, using **both methods**, **8 replicate samples** for **each level** of contamination, giving a total of 24 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
1	128	96	88	88	88	0	0
2	128	96	88	0	0	88	88
3	128	96	88	0	0	88	88

** Two laboratories did not receive the samples.*
*** The results of one laboratory were excluded because of incorrect use of the protocol of the alternative method.*

Calculations

- Relative accuracy (AC) = 100%
- % specificity (SP) = 100%
- % sensitivity (SE) = 100%

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) =100%	(PA + ND) / (PA + PD + ND) = 100%

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 and the reference method:**

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

Conclusion

Variability of the alternative method (accordance, concordance, concordance odds ratio) is identical 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