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NordVal International Certificate

Issued for:	BAX® System PCR Assay for *Salmonella 1 and Salmonella 2* (Classic + Q7 instruments)
NordVal No:	030
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Valid until:	1 May 2020

BAX® System PCR Assay for *Salmonella* (Classic + Q7 instruments)

Manufactured by:
Qualicon Diagnostics LLC,
A Hygiena Company
200 Powder Mill Road
Wilmington, DE 19803 USA

Supplied by:
OXOID Limited
Thermo Fisher Scientific,
Wade Road, Basingstoke,
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fulfils the requirements of the NordVal validation protocol 1 / ISO 16140-2:2016. The reference method was EN ISO 6579:2002 / EN ISO 6579-1:2017: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. The validations have been carried out by the expert laboratories Institut Pasteur de Lille and ADRIA Développement, France.

The results document that the BAX® System PCR Assay for *Salmonella* provides equivalent results to the reference method.

Date: 1. May 2018

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

PRINCIPLE OF THE METHOD

The BAX® System for detection of *Salmonella* is a detection kit using PCR (Polymerase Chain Reaction) technology. The method procedure consists of the following four steps:

- √ Enrichment
- √ DNA extraction lysis
- √ Amplification
- √ Detection

The Bax® System for detection of Salmonella is targeting a specific bacterial DNA fragment, which is specific for *Salmonella* and which is not present in any other bacteria, and hence is an indicator of the presence of *Salmonella*.
The PCR allows the BAX® System to realize a specific and rapid amplification of the DNA. After the lysis step, the Bax® System cycler/detector is doing both amplification and automated detection.

Enrichment:

- For all products, except dairy products, raw meat and raw poultry:
  Pre-enrichment: 16 h - 20 h at 37°C in BPW (d 1/10)
  Subculture: 3 h – 4 h in BHI (10 µl BPW/500 µl BHI)
- Raw poultry and raw meat (non seasoned):
  Pre-enrichment: 16 h - 20 h at 37°C in prewarmed BPW (d 1/10)
- Dairy products (except milk powders): Pre-enrichment: 20 h - 24 h at 42°C in BPW supplemented with Novobiocin (20 mg/l) (d 1/10)
- Raw meat (seasoned or not) Pre-enrichment: 24 h at 42°C in MP broth
- Raw beef meat (seasoned or not) Pre-enrichment: 9 h – 24 h at 42°C in prewarmed MP broth

DNA extraction lysis:

- Addition of 5 µL enrichment broth to 200 µL lysis reagent
- 20 minutes at 37°C, 10 minutes at 95°C and Cool at 2-8°C for 5 min

Amplification:

- transfer 50 µL of the lysate in a PCR tube and run the PCR in the automate

Detection:

- The fluorescence is measured directly by the BAX® system, which provides positive or negative results.
- By following the conventional testing methods described in the reference method, including a purification step.
- By streaking the last enriched media on a selective agar plate and by applying the tests described in the reference method on typical colonies.
  For raw meats enriched in the BAX® System MP media, transfer MP media into RVS (incubation for 24 h ± 3 h at 41.5°C), followed by isolation on *Brilliance*TM Salmonella agar plate and confirmation of typical colonies by a latex test.

FIELD OF APPLICATION

The method is applicable for the detection of *Salmonella* spp. in a broad range of foods, animal feed and environmental samples.

VALIDATION HISTORY

Studies have been carried out by Institut Pasteur de Lille in 2002, 2004 and 2006 and by ADRIA Développement, France in 2008-2017. The latest study in 2017/2018 was an extension study for a modification of the software as well as an update to comply with ISO 16140-2:2016.
In 2010, a minor modification was performed by adding the hot start functionality to the BAX® Salmonella test kit (then designated BAX® Salmonella 2 test kit). Results have demonstrated, that the modification has no impact on the analytical performances of the kit.

SENSITIVITY STUDY

A number of products, both naturally and artificially contaminated, have been tested. The matrices tested belong to the following categories: Dairy products, meat, fish, vegetables, pastries, egg products, ready to eat meals, animal feed and environmental samples.

The comparison studies show that there are no significant differences between the results obtained by using this alternative method or the reference method.

A total of 749 samples are included in the study, whereof 539 samples are from the previous validations. The samples were analysed with both the alternative method and the reference method. The results are given in Table 1.

Table 1: Sensitivity study

Category	PA	NA	ND	PD	N	PPND	PPNA	SE_{alt} (%)	SE_{ref} (%)	RT (%)	FPR (%)	Kappa
Composite	36	40	1	0	77	0	0	97.3	100	98.7	0	0.97
Meat products	21	33	5	9	68	0	0	85.7	74.3	79.4	0	0.68
Dairy products	27	41	1	7	76	0	0	97.1	80.0	89.5	0	0.78
Vegetables and seafood	28	35	2	0	65	0	1	93.3	100	97	2.8	0.94
Egg products and ingredients	29	50	1	0	80	0	0	96.7	100	98.8	0	0.97
Feed	29	36	1	0	66	0	0	96.7	100	98.5	0	0.97
Environmental	30	40	1	0	71	0	0	96.8	100	98.6	0	0.97
Raw meat MP 24 h	21	33	7	5	66	0	0	78.8	84.8	81.8	0	0.62
Raw beef MP 9 h	33	45	6	6	90	0	0	86.7	86.7	86.7	0	0.75
Raw beef MP 24 h	37	44	2	7	90	0	0	95.7	84.8	90.0	0	0.80
Total, with raw beef MP 9 h	**254**	**353**	**27**	**25**	**659**	**0**	**1**	**91.8**	**91.2**	**92.1**	**0.3**	**0.83**
Total, with raw beef MP 24 h	**258**	**352**	**28**	**21**	**659**	**0**	**1**	**93.2**	**90.9**	**92.6**	**0.3**	**0.84**

PA = number of obtained results that are positive with the alternative and the reference method that are confirmed positives
NA = number of obtained results that are negative with both the alternative and the reference method
ND = number of obtained results that are negative with the alternative method and positive with the reference method (false negative)
PD = number of obtained results that are positive with the alternative method and negative with the reference method and confirmed as positive.
PPNA = presumptive positive of the alternative method which after confirmation was found to be negative – the

reference method was negative (false positive without confirmation)
PPND = presumptive positive with both method but confirmed as negative
SEalt = sensitivity for the alternative method
SEref = sensitivity for the reference method
RT = relative trueness
FPR = false positive ratio for the alternative method

There were 27 negative deviations (ND); results that were negative with the alternative method and positive with the reference method, which were confirmed as positives. There were 34 positive deviations (PD), i.e. results that were positive with the alternative method and negative with the reference method. Most of the deviations are for meat products. For meat products, the study is unpaired (having different enrichment procedures) and as there are more positive deviations than negative deviations, the acceptance criteria for the difference between ND and PD is fulfilled.

The statistical entity Kappa shows that there is not a very good agreement between the alternative method and the reference method (Kappa <0.8) for meat products due to deviations. For the total number of analysis, the statistical entity Kappa shows that there is a very good agreement between the results obtained by the alternative method and the reference method.

Relative level of detection

One type for the eight different matrix categories were analysed at the different levels

- Level 1: 0 CFU/g or ml,
- Level 2: level necessary to obtain 0 to 50 % positive,
- Level 3: level necessary to obtain 50 to 75 % positive,
- Level 4: level necessary to obtain 100 % positive.

The relative level of detection (RLOD) for the alternative method relative to the reference method should be no more than 2.5 for unpaired studies (different incubation steps) and no more than 1.5 for paired studies. All matrices had satisfactory RLOD.

Level of Detection

The data used for the calculation of RLOD have been used to calculate an estimate for the level of detection for the alternative method. This has been done by using the *EXCEL program for the estimation of the POD function and the LOD of a qualitative microbiological measurement method* according to Wilrich, C., and P.-Th. Wilrich: AOAC International **92** (2009) 1763 - 1772.

Protocol 1	Level cfu / 25g (ml)	Number of positives/ replicates	LOD_{50} (cfu/25g)	LOD_{50} Lower confidence level (cfu/25g)	LOD_{50} Upper confidence level (cfu/25g)
Raw milk	0 0.5 0.96 2.00 2.64	0/6 2/6 5/6 5/6 6/6	0.7	0.4	1.2
Oysters Seafood	0 0.42 0.80	0/6 3/6 4/6	0.4	0.2	0.7

Protocol 1	Level cfu / 25g (ml)	Number of positives/ replicates	LOD_{50} (cfu/25g)	LOD_{50} Lower confidence level (cfu/25g)	LOD_{50} Upper confidence level (cfu/25g)
	1.40	6/6			
Cod fillet	0 0.29 0.40 0.70	0/6 2/6 5/6 6/6	0.2	0.1	0.4
Ground beef	0 0.09 0.18 0.36 0.90 2.63	0/6 0/6 1/6 3/6 5/6 5/6	0.6	0.3	1.1
Mayonnaise based deli-salad	0 0.70 1.00	0/5 8/20 5/5	0.7	0.4	1.2
Ground pork	0 0.40 2.90	0/5 7/20 4/5	0.8	0.4	1.6
Combined result			0.6	0.4	0.7

The Combined LOD50 is 0.6 cfu/25g

Inclusivity / exclusivity

Inclusivity: 471 strains of *Salmonella* spp. were detected out of the 473 tested.

Exclusivity: The study of 150-non-*Salmonella* spp showed no positive results.

INTERLABORATORY STUDY:

The interlaboratory study was conducted in 2006.
Number of participating laboratories: 12
Number of laboratories reporting results: 11
The analyses were performed on samples of pâté, artificially contaminated with a strain of *Salmonella* Typhimurium isolated from pork liver at the following three contamination levels:

- 0 cfu/g
- 3 cfu/g
- 30 cfu/g

The laboratories analysed 8 replicates for each level using both the alternative and the reference method. The following results were obtained:

- Sensitivity: 100%
- Specificity: 100%
- Relative accuracy: 100%
- Kappa: 1.0

The difference and the sum of the negative and positive deviations were lower than the acceptability limit (AL).

The results of the interlaboratory study are satisfactory.

CONCLUSION

By the comparison study and the interlaboratory study according to ISO 16140-2, it is documented that the alternative method provides equivalent results to the reference method.