NordVal International / NMKL
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NordVal International Certificate

Issued for:	iQ-Check® *Salmonella* II kit
NordVal No:	038
First approval date:	10 October 2009
Extention date:	31 December 2017
Valid until:	31 December 2019

iQ-Check® *Salmonella* II kit

Manufactured by:
Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive,
Hercules, California,
94547- USA

Supplied by:
Bio-Rad Laboratories,
3 Blvd Raymond Poincare,
92430 Marnes-la-Coquette,
France

fulfils the requirements of the NordVal Validation Protocol. The reference method was EN ISO 6579:2002 – Food microbiology -Horizontal method for the detection of *Salmonella* spp.

NordVal International has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratories I'Institut Pasteur de Lille and ADRIA Développement, France, respectively. The validations have been carried out according to ISO 16140:2003 with additional studies for the inclusivity/exclusivity tests. The calculations of the sensitivity acceptance criteria are carried out according to ISO 16140-2:2016. NordVal International concludes that it has been satisfactorily demonstrated that results document no difference in the performances between the iQ-Check® *Salmonella* II kit and the reference method. Further, it was demonstrated that confirmation is not necessary if not required according to legislation.

Date: 20 December 2017

Yours sincerely

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

PRINCIPLE OF THE METHOD

The iQ-Check *Salmonella* II is a qualitative method allowing the detection of *Salmonella* spp specific DNA sequences after enrichment by culture in buffered peptone water. It is based upon polymerase chain reaction and real time detection using fluorescent probes.

iQ-Check *Salmonella* II describes the following four procedures, differing from each other in preliminary enrichment and lysis steps:

- Standard Protocol I: 18h ± 2h at 37°C ± 1°C enrichment in buffered peptone water followed by the standard lysis protocol.
- Easy Protocol I in micro plates: 21 h ± 1h at 37°C ± 1°C enrichment in buffered peptone water, followed by a simplified extraction protocol, no longer requiring the first centrifugation step.
- Standard Protocol II: specific for raw meat: 10h ± 2h at 37°C ± 1°C enrichment in buffered peptone water followed by the standard lysis protocol.
- Easy Protocol II in microplates, specific for raw beef: 21 h ± 1h at 37°C ± 1°C enrichment in buffered peptone water, followed by a simplified extraction protocol, no longer requiring the first centrifugation step.
- Easy Protocol II, specific for meat products, 18h ± 2h at 37°C ± 1°C enrichment in buffered peptone water followed by the standard lysis protocol.

VALIDATION HISTORY

The expert laboratories I'Institut Pasteur de Lille and ADRIA Développement, France carried out extensive studies in 2007 and 2008.

For the 2011 renewal: In addition of the four previously validated NordVal protocols for DNA extraction, the following changes were approved:

- a modification of the extraction of DNA from meat products, using a new "Deepweell plate" was introduced. The expert laboratory ADRIA Développement, France had provided comparative data for the use of the new extraction protocol. The data showed that the extraction modification had no impact on the analytical result
- a new protocol of extraction for meat products, Easy Protocol II, 18h ± 2h was validated by ADRIA.

For the 2017 renewal, the obtained results have been evaluated according to the acceptance criteria of ISO 16140-2:2016.

FIELD OF APPLICATION

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

COMPARISON STUDY

COMPLIANCE BETWEEN iQ-CHECK *SALMONELLA* II AND THE REFERENCE METHOD:

In 2007 and 2008 the expert laboratories carried out extensive study on 582 product samples, including 64 naturally contaminated, 219 artificially contaminated and 299 non-contaminated, belonging to the following main food categories:

Meat products, dairy products, fish-based and vegetable products, egg products, animal feed, environmental samples
All samples were analysed in single by both the alternative and the reference method.

The comparison studies show that there are no significant differences between the results obtained by using one of the four protocols of the alternative method and the reference method. The following results were obtained:

Accuracy, sensitivity, specificity

Standard Protocol I

Results after screening

Matrices	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	29	30	1	0	60	98,3%	96,7%	100,0%	0,97
Dairy products	43	39	2	4	88	93,2%	95,6%	90,7%	0,86
Fish based and vegetable products	32	38	0	1	71	98,6%	100,0%	97,4%	0,92
Egg products	29	30	0	2	61	96,7%	100,0%	93,8%	0,93
Animal feed	30	33	0	0	63	100,0%	100,0%	100,0%	1,00
Environment samples	28	52	2	2	84	95,2%	93,3%	96,3%	0,90
Total	191	222	5	9	427	**96,7%**	**97,4%**	**96,1%**	**0,93**

* see definitions of the abbreviations on page 4.

Results after Confirmation

Matrices	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	29	30	1	0	60	98,3%	96,7%	100,0%	0,97
Dairy products	43	41	2	2	88	95,5%	95,6%	95,3%	0,91
Fish based and vegetable products	32	39	0	0	71	100,0%	100,0%	100,0%	1,00
Egg products	29	31	0	1	61	98,4%	100,0%	96,9%	0,97
Animal feed	30	33	0	0	63	100,0%	100,0%	100,0%	1,00
Environment samples	28	54	2	0	84	97,6%	93,3%	100,0%	0,95
Total	191	228	5	3	427	**98,1%**	**97,4%**	**98,7%**	**0,96**

* see definitions of the abbreviations on page 4.

The Acceptability Limits (AL) for the sensitivity for six categories are ND-PD≤ 6 and ND+PD ≤ 16, respectively.
For Standard Protocol I, screening: ND-PD = 5-9= -4 and ND+PD = 5+9=14
For Standard Protocol I, confirmation: ND-PD = 5-3= -2 and ND+PD = 5+3=8

NordVal International requires that there should be a very good agreement between the methods, i.e. kappa > 0.80.

The acceptance criteria are fulfilled for Standard Protocol I.

Easy Protocol I

Results after screening

Matrices	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	29	29	1	1	60	96,7%	96,7%	96,7%	0,93
Dairy products	42	39	3	4	88	92,0%	93,3%	90,7%	0,84
Fish based and vegetable products	30	35	2	4	71	91,5%	93,8%	89,7%	0,83
Egg products	29	29	0	3	61	95,1%	100,0%	90,6%	0,90
Animal feed	29	30	1	3	63	93,7%	96,7%	90,9%	0,87
Environment samples	29	46	1	8	84	89,3%	96,7%	85,2%	0,78
Total	188	208	8	23	427	**92,7%**	**95,9%**	**90,0%**	**0,85**

* see definitions of the abbreviations on page 4.

Results after confirmation

Matrices	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	29	30	1	0	60	98,3%	96,7%	100,0%	0,97
Dairy products	42	42	3	1	88	95,5%	93,3%	97,7%	0,91
Fish based and vegetable products	30	39	2	0	71	97,2%	93,8%	100,0%	0,94
Egg products	29	31	0	1	61	98,4%	100,0%	96,9%	0,97
Animal feed	29	32	1	1	63	96,8%	96,7%	97,0%	0,94
Environment samples	29	54	1	0	84	98,8%	96,7%	100,0%	0,97
Total	188	228	8	3	427	**97,4%**	**95,9%**	**98,7%**	**0,95**

* see definitions of the abbreviations on page 4.

The Acceptability Limits (AL) for the sensitivity for six categories are ND-PD≤ 6 and ND+PD ≤ 16, respectively.
For Easy Protocol I, screening: ND-PD = 8-23= -15 and ND+PD = 8+23=31
For Easy Protocol I, confirmation: ND-PD = 8-3= 5 and ND+PD = 8+3=11

NordVal International requires that there should be a very good agreement between the methods, i.e. kappa > 0.80.

The acceptance criteria are fulfilled for Easy Protocol I.

Standard Protocol II (raw meat)

Results after screening

Matrix	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Raw meat	51	36	1	1	89	97,8%	98,1%	97,3%	0,95

* see definitions of the abbreviations below.

Results after confirmation

Matrix	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Raw meat	51	37	1	0	89	98,9%	98,1%	100,0%	0,98

* see definitions of the abbreviations below.

Easy Protocol II (raw beef)

Results after screening

Matrix	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Raw beef	31	34	1	0	66	98,5%	96,9%	100,0%	0,97

* see definitions of the abbreviations below.

The overall sensitivity and the agreement between the methods are satisfactory, and hence confirmation is not required. The results after confirmation were however identical.

Easy Protocol II (meat products)

In 2011, a total of 67 meat samples were analysed 17 naturally contaminated, 13 artificial contaminated and 37 non-contaminated samples. The following results were obtained:

Matrix	* PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Meat products	30	37	0	0	67	100,0%	100,0%	100,0%	1,0

* see definitions of the abbreviations below.

The overall sensitivity and the agreement between the methods are satisfactory, and hence confirmation is not required.

PA = number of obtained results that are positive with both the alternative and the reference method

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 (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)

Relative AC = The relative accuracy; the degree of correspondence between the response obtained by the alternative method and the reference method.

Relative SE = The relative sensitivity; the ability of the alternative method to detect the analyte compared to the reference method

Relative SP = The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

Kappa = The degree of agreement between the alternative method and the reference method, kappa of 0,80 or higher is considered to be very good agreement.

The Acceptability Limits (AL) for the sensitivity for one category are ND-PD≤ 3 and ND+PD ≤ 6, respectively.

NordVal International requires that there should be a very good agreement between the methods, i.e. kappa > 0.80.

The acceptance criteria are fulfilled for all protocols.

A study conducted in 2004 on 362 samples on different matrices showed satisfactory results for iQ-Check Salmonella. The following was obtained:

- Relative accuracy: 98.9%
- Relative specificity: 100.0%
- Relative sensitivity: 97.5%
- Agreement between the methods, kappa: 0,98

Detection Level

The different matrices have been analysed 6 times at 4 different contamination levels by both methods. The limit of detection was found to be 1-10 cfu in a sample of 25g or 25 ml for all matrices and method protocols.

Inclusivity /exclusivity

The studies carried out in 2007 and 2008, respectively, for the alternative method showed that:

- 156 strains of *Salmonella* were detected out of the 156 tested, regardless of the lysis protocol used.
- The study of 30 non-*Salmonella* strains resulted in no cross-reactions regardless of the lysis protocol used.

The study carried out in 2004 for the alternative method showed that:

- 51 strains of Salmonella were detected out of the 51 tested, regardless of the protocol used.
- The study of 31 non-Salmonella strains resulted in no cross reactions, regardless of the protocol used.

COLLABORATIVE STUDY:

The collaborative study was conducted in 2008 using Easy Protocol I.
Number of participating laboratories: 19

Results from 8 laboratories were excluded due to intralaboratory contamination of the samples, and one laboratory received the samples too late and hence unable to perform the tests.

The analyses were performed on samples of pasteurized milk, artificially contaminated with a strain of *Salmonella typhimurium* at the following three contamination levels:

- 0 cfu/25 ml
- 1-10 cfu/25 ml
- 5-50 cfu/25 ml

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

- Sensitivity: 100%
- Specificity: 95%
- Relative accuracy: 100%
- Kappa: 1,00

Thus, there is no statistical difference between the results obtained by the two methods.

CONCLUSION:

According to the comparison and the collaborative study no statistical differences were found between the iQ-check *Salmonella* II test and the reference methods, ISO 6579:2002, for the detection of *Salmonella* in foods, animal feeds and environmental samples. Further, it was demonstrated that confirmation is not necessary for neither of these method protocols.