

NordVal Certificate

Issued for:	foodproof[®] <i>Salmonella</i> Detection Kit, Hybridization Probes and foodproof[®] <i>Salmonella</i> Detection Kit, 5' Nuclease in combination with foodproof[®] ShortPrep I Kit
NordVal No:	023
First approval date:	28 October 2005
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Valid until:	10 June 2013

foodproof[®] *Salmonella* Detection Kit, Hybridization Probes and foodproof[®] *Salmonella* Detection Kit, 5' Nuclease in combination with foodproof[®] ShortPrep I Kit

Manufactured and supplied by:
BIOTECON Diagnostics GmbH,
Hermannswerder 17,
14473 Potsdam, Germany.


fulfils the requirements of the NordVal validation protocol. The reference method was EN ISO 6579:2002 for detection of *Salmonella* spp. in food, animal feed and environmental samples.

NordVal has reviewed the method and the validation studies conducted by the MQD, Institute for Analytic and Hygiene in Güstrow, Germany. NordVal has studied the enclosures to the application and evaluated the results obtained in the validations. The results document no statistical differences in the performances between the alternative method and the reference method. NordVal has concluded that it has been satisfactorily demonstrated that the requirements for the sensitivity and the agreement between the methods are fulfilled, further, that confirmation of obtained positives are not necessary.

Date: 8 June 2011

Yours sincerely


Sven Qvist
Chair of NordVal


Hilde Skaar Norli
NMKL Secretary General

PRINCIPLE OF THE METHOD

The principle is real-time PCR and detection with specific, fluorescence labelled probes.

After DNA isolation using the **foodproof**[®] ShortPrep I Kit (Art. No. S 400 01), designed for the rapid preparation of bacterial DNA for direct use in PCR, the real-time detection of *Salmonella* DNA is carried out either by using the **foodproof**[®] *Salmonella* Detection Kit, Hybridization Probes or the **foodproof**[®] *Salmonella* Detection Kit, 5'Nuclease (Art. No. R 302 27).

For food and feed samples inoculate 25 g. For environmental samples inoculate an area of 100 cm². Perform the pre-enrichment according to EN ISO 6579. The detection kit provides all the reagents required for the PCR.

FIELD OF APPLICATION

The **foodproof**[®] *Salmonella* Detection Kit, Hybridization Probes and the **foodproof**[®] *Salmonella* Detection, 5'Nuclease in combination with **foodproof**[®] ShortPrep I Kit for the detection of *Salmonella* are intended for the detection of *Salmonella* DNA isolated. The methods are tested on foods, feeds and environmental samples.

VALIDATION HISTORY

The **foodproof**[®] *Salmonella* Detection Kit, Hybridization in combination with **foodproof**[®] ShortPrep I Kit was first approved in 2005 based on a comparison study and a collaborative study.

In 2011, the method was modified: A new system was devolved using hydrolysis probes instead of hybridisation probes. The modification, using a new primer, required a new comparison study of the selectivity (inclusivity and exclusivity) and a comparison study of the relative accuracy to measure the degree of correspondence between the results obtained by the **foodproof**[®] *Salmonella* Detection kit and the reference method. As the method procedure was unchanged, NordVal did not require an additional collaborative study.

METHOD PERFORMANCE CHARACTERISTICS

Selectivity: Inclusivity/exclusivity

The relative selective was satisfactory in the study approved in 2005.

The additional study approved in 2011:

Inclusivity: All the 54 isolates from *Salmonella* spp. were positively detected.

Exclusivity: All the 30 bacteria from *Enterobacteriaceae* and other closely related bacteria or organisms of the same habitat were negative.

The selectivity, i.e. the inclusivity and exclusivity, was 100%.

Relative accuracy, relative sensitivity and relative specificity:

The results of the study carried out in 2005 were satisfactory.

The results of study approved in 2011:

Three different sub matrices of meat (minced meat, poultry meat, salami), feed (fish feed, bone meal, chew sticks for dogs) and environmental samples (metal surface, glass surface, plastic surface), respectively, were included in the study. Two strains relevant for each matrix were selected. Three inoculation levels were used: 0 = negative control, 1-10 cells per 25 g / area of 100 cm² sample and 10-100 cells per 25 g / area of 100 cm² sample. For each ma-

trix 60 samples were analysed.

The following results were obtained:

Table 1: Results after screening

Matrix	*					Relative AC	Relative SE	Relative SP	Kappa
	PA	NA	ND	PD	Sum	%	%	%	
Meat	39	21	0	0	60	100	100	100	1.0
Environmental	21	39	0	0	60	100	100	100	1.0
Feed	32	28	0	0	60	100	100	100	1.0
Total	92	88	0	0	180	100	100	100	1.0

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.

It was confirmed that the results obtain did not contain any false positives nor false negatives.

Detection Level

The limit of detection is 1-10 cells per 25 g, which was obtained both with the alternative method and the reference method for all matrices.

CONCLUSION

The **foodproof**[®] *Salmonella* Detection Kit, Hybridization Probes and the **foodproof**[®] *Salmonella* Detection, 5'Nuclease in combination with **foodproof**[®] ShortPrep I Kit for the detection of *Salmonella* perform equivalent to the reference method.