



NordVal Certificate

Issued for:	<i>Salmonella</i> detection method by real-time PCR
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Salmonella detection method by real-time PCR


Danish Technological Institute, Danish Meat Research Institute, Maglegaardsvej 2, DK 4000 Roskilde, Denmark, has applied for a renewal of the NordVal certification on a rapid *Salmonella* detection method based on a novel real-time PCR-assay. The method is applicable for raw meat (including poultry neck skin) swabs from cattle and pig carcasses, and fecal samples (poultry boot swabs). The performance of this PCR method has been compared to the following reference method:

- ✓ NMKL 71, 5th Ed., 1999: *Salmonella* spp.. Detection in foods. (Raw meat and swabs)
- ✓ NMKL 187, 2007: *Salmonella* spp.. Detection in foods, faeces and materials from primary animal production using MSRV. (Poultry faeces on sock samples)

The results document no statistical difference in the performances between the methods.

Date: 01.11.2010

Yours sincerely


Sven Qvist
Chair of NordVal


Hilde Skår Norli
NMKL Secretary General



PRINCIPLES OF THE METHOD

The method is based on a shortened pre-enrichment in buffered peptone water for 10-16 hours. A 1 or 5 mL sub sample is centrifuged in 200 µL buffer. DNA is extracted by use of the KingFisher system and the "MagneSil KF Genomic System". Subsequently, *Salmonella* is detected by a novel real time PCR assay. The assay is a 5' nuclease (TagMan) real-time PCR developed for the specific detection of *Salmonella*.

The method had been developed by the National Food Institute of the Technical University of Denmark, as a 14 hours method for the detection of *Salmonella* in raw meat, 16 hours for detection of *Salmonella* in swabs of carcasses, and 20 hours of the detection of *Salmonella* on sock samples with poultry faeces.

FIELD OF APPLICATION

The method is validated on raw meat, swabs from beef and pork carcasses and poultry faeces on sock samples (also named boot swabs). This *Salmonella* real-time PCR method can be used without further confirmation, i.e. if it is not required according to legislation.

RELIABILITY OF THE METHOD

Comparison studies and collaborative studies have been organised by the National Food Institute of the Technical University of Denmark in 2007. The KingFisher and real-time PCR method was compared against the reference methods; NMKL 71 and NMKL 187. The following results were obtained:

Table 1: The KingFisher and real-time PCR method was compared against the reference methods; NMKL 71 and NMKL 187

Matrix	Raw meat	Carcass swabs	Poultry faeces
Limit of Detection	1-10 cfu/ 25 g	1-10 cfu/ 25 g	10-100 cfu/ 25 g
Relative accuracy	99%	99%	100%
Relative sensitivity	99%	95%	100%
Relative specificity	100%	100%	100%
Relative selectivity	100%	100%	100%

The selectivity, inclusivity and exclusivity, was tested with satisfactory results for the DNA extraction using KingFisher extraction and real-time PCR (Food Appl. Environ. Microbiol. 70, 7046-7052). The performances of these characteristics are considered to be valid for the method modification described in this NordVal validation.

CONCLUSION

The results of the studies are satisfactory. There is no statistical difference between the alternative method and the reference methods.