



NordVal Certificate

Issued for:	BAX Q7 <i>Campylobacter jejuni, coli</i> and <i>lari</i> in chicken cloacae swabs
NordVal No:	39
First approval date:	10 October 2009
Valid until:	10 October 2011

BAX® Q7 *Campylobacter jejuni, coli* and *lari* in chicken cloacae swabs

Manufactured by:
DuPont Qualicon
ESL Building 400, PO Box 80400
Bedford Bldg.
3531 Silverside road
Wilmington, DE 19810 USA

Supplied by:
Oxoid A/S, ThermoFisher
Scientific
Lunikvej 28, 2970 Greve.
Denmark


fulfils the requirements of the NordVal validation protocol. The reference method was EN ISO:10272-1:2006: Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 1: Detection method.

NordVal has studied the enclosures to the application and evaluated the results obtained in the validations conducted by the expert laboratory Danish Veterinary and Food Administration, Denmark. The studies were conducted according to the NordVal protocol. The results document no statistical difference in the performances between the BAX Q7 method and the reference method in the BAX Q7 in chicken cloacae swabs for levels above 100 cfu/g. 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: 22 February 2010

Yours sincerely


Sven Qvist
Chair of NordVal


Hilde Skaar Norli
NMKL Secretary General



PRINCIPLE OF THE METHOD:

The method is a direct method without enrichment step. The results are available within ca 3 hours. The sensitivity is 100 CFU/ml.

Lysis reagent was prepared by adding 150 µl protease to a 12 ml bottle of lysis buffer. For each sample 50 µl sample was added to 200 µl prepared lysis reagent in cluster tubes.

Tubes were lysed for 20 minutes at 37 ±2° C and inactivated in 10 minutes at 95 ± 3° C followed by 5 minutes in a cooling block.

PCR tubes were arranged in a rack, and 30 µl lysate was added to the tubes for PCR processing in the BAX System Real-Time PCR assay for *Campylobacter jejuni/coli/lari*.

FIELD OF APPLICATION:

The method is applicable for the detection of *C. jejuni*, *C. coli* and *C. lari* in chicken cloacae swabs.

COMPARISON STUDY

The detection level is higher for Bax Q7 than the reference method. The detection level for BAX Q7 is about 100 cfu/g, for the reference method the limit of detection is between 1-10 cfu/g (≈2 cfu/g).

For levels above 100 cfu/g the sensitivity is satisfactory, and so is the agreement between the methods. The relative specificity and accuracy is satisfactory.

At levels where the Bax Q7 is applicable, it performs equivalent for *Campylobacter jejuni* as the reference method.

Accuracy, sensitivity, specificity

60 chicken faeces naturally contaminated, 30 positive and 30 negative was analysed in 2009, with the following results. The study is conducted on *Campylobacter jejuni*.

Results after screening

Matrix	PA	NA	ND	PD	Sum	Relative AC	Relative SE	Relative SP	Kappa
Cloacae swabs	30	30	0	0	60	100%	100%	100%	1,00

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 agreement between the two methods is very good (Kappa > 0,80) for all matrices, and



the overall sensitivity is satisfactory ($\geq 95\%$). The same result was obtained for the confirmation.

Detection Level

The limit of detection for the ISO method is between 1-10 cfu/g (about 3 when estimated according to trimmed Spearman-Kärber) and for the Bax method it is closed to 100 cfu/g, (95 cfu/g when estimated according to trimmed Spearman-Kärber). The detection level is higher for Bax Q7 than the reference method, due to smaller sample volume.

For levels above 100 cfu/g the sensitivity is satisfactory, and so is the agreement between the methods. The relative specificity and accuracy is satisfactory.

Inclusivity/exclusivity

The Inclusivity/exclusivity tests performed at the expert laboratory Cherney Microbiological Services, Green Bay, Wisconsin USA for AOAC Research Institute in 2007 show that the selectivity, i.e. the inclusivity and exclusivity is satisfactory for *Campylobacter jejuni*, *coli* and *lari* in corresponding matrices.

COLLABORATIVE STUDY:

The collaborative study was conducted in 2009 on *Campylobacter jejuni*.

Number of laboratories reporting results: 7

The following results were obtained:

- Sensitivity: 100% for levels above the LOD of the Bax Q7 method
- Specificity: 100%
- Relative accuracy: 100%
- Kappa: 1,00 for levels above LOD. For levels below LOD, Kappa < 0,80

Conclusion:

The detection level is higher for Bax Q7 than the reference method, due to smaller sample volume. The detection level for BAX Q7 is about 100 cfu/g,

For levels above 100 cfu/g the Bax Q7 performs equivalent as the reference method.

It was noted that the comparison and the collaborative study were performed on *C. jejuni* only. However, as *C. jejuni* is the most frequent strain (90-95%) in faeces, and the selectivity tests show that the method is applicable for *C.coli* and *C. lari* as well, and hence the method is approved for all three strains. The studies showed that confirmation is not necessary.