NMKL - NordVal International
c/o Institute of Marine Research
P.O. box 1870 Nordnes, N-5817 Bergen, Norway
www.nmkl.org

NordVal International Certificate

Issued for:	BAX® System Real-Time PCR Assay *Campylobacter jejuni/coli/lari*
NordVal No:	039
First approval date:	10 October 2011
Renewal date:	24 November 2021
Valid until:	01 December 2023

BAX® System Real-Time PCR Assay Campylobacter jejuni/coli/lari

Manufactured and supplied by:

Hygiena, LLC
941 Avenida Acaso,
Camarillo, CA 93012 , USA
Phone:1-800-863-6842
Email: Diagnostics.support@hygiena.com

fulfils the requirements of the NordVal Validation Protocol. The performance of the BAX® System Real-Time PCR Assay *Campylobacter jejuni/coli/lari* has been compared against the reference method: 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. The reference method of 2006 is technical equivalent to the EN ISO 10272-1:2017.

The validation studies are carried out in accordance with ISO 16140-2:2016 and the NordVal International protocol. The results document that the reference method and the alternative method perform equivalent for poultry faeces on cloacae swabs for levels above 100 cfu/g.

The production of the kits is fulfilling the requirements given in ISO 9001.

Date: 24 November 2021

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Secretary General

PRINCIPLE OF THE METHOD

The method is a direct method without enrichment step.

The BAX® system uses the Polymerase Chain Reaction (PCR) to amplify specific DNA fragments, which are stable and unaffected by growth conditions. Each fragment is a genetic sequence that is unique to the targeted organisms, thus providing a highly reliable indicator that the organisms are present. The BAX® system simplifies the PCR process by combining the requisite PCR reagents into a stable dry manufactured tablet already packaged inside the PCR tubes. The BAX® system Q7 instrument uses multiple filters to measure signal at the end of each cycle and reports results for each target in less than 90 minutes.

In the method performance study, the lysis reagents were prepared by adding 150 µl protease to 12 ml bottles of lysis buffer. After collection of 2 cloacae swabs (2 cotton swabs are used for cloacae sampling in 25 chickens [1 cotton swab then represents 12 or 13 chickens]), the swabs were placed into vial containing 2 mL of 0.9% sodium chloride and 0.1% peptone. For each test sample, 50 µl of the sample were added to 200 µl prepared lysis reagent in cluster tubes. The tubes were lysed for 20 minutes at 37 ±2° C, inactivated for 10 minutes at 95 ±3° C followed by 5 minutes in a cooling block.

The 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 *Campylobacter jejuni/coli/lari*.

The results were available within 3 hours.

FIELD OF APPLICATION

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

HISTORY

The comparison study was carried out by the Danish Veterinary and Food Administration, Region North in 2009 and the Inclusivity/exclusivity tests were performed at the at the expert laboratory Cherney Microbiological Services, Green Bay, Wisconsin USA for AOAC Research Institute in 2007 The collaborative study was conducted in 2009.

COMPARISON STUDY

Accuracy, sensitivity, specificity

60 chicken faeces naturally contaminated, 30 positive with *Campylobacter jejuni* and 30 negative with the following results:

Results of the sensitivity study

Matrix	PA	NA	PD	ND	FP	Sum	Relative Trueness RT	Sensitivity alternative method SE $_{alt}$ (%)	Sensitivity reference method SE $_{ref}$ (%)	FPR
Cloacae swabs	30	30	0	0	0	60	100%	100%	100%	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.

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

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

FP = False positive test result. Positive result by the tested method that is actually confirmed as a negative result

FPR = False positive ratio.

The agreement between the alternative and the reference method is very good (Kappa > 0.80).

Detection Level

The limit of detection for the ISO method is between 1-10 cfu/g (about 3 when estimated according to trimmed Spearman-Karber) and for the BAX® method it is closed to 100 cfu/g, (95 cfu/g when estimated according to trimmed Spearman-Karber). The detection level is higher for BAX® Q7 than the reference method, due to smaller sample volume of the BAX® assay and because samples pass through an enriched step during the ISO reference method.

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

BAX ® system inclusivity results were 100% accurate for 52 Campylobacter strains of the target species (18 *C. jejuni*, 15 *C. coli*, and 19 *C. lari*). Exclusivity results were 100% accurate for 38 non-target strains. Thus, the inclusivity and exclusivity are satisfactory for Campylobacter *jejuni, coli* and *lari* in corresponding matrices.

INTERLABORATORY STUDY

The interlaboratory study was conducted 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: 1.0 for levels above LOD. For levels below LOD, Kappa < 0.80

CONCLUSION

The detection level is higher for BAX® Q7 instrument 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 interlaboratory 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.