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# NordVal International Certificate

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| Issued for: | BAX® System Real-Time PCR Assay for *Campylobacter jejuni / coli* and *lari* |
| NordVal No: | 039 |
| First approval date: | 10 October 2011 |
| Renewal date: | 1 December 2017 |
| Valid until: | 1 December 2019 |

## BAX® System Real-Time PCR Assay for *Campylobacter jejuni / coli* and *lari*

Manufactured by:
Qualicon Diagnostics LLC
Experimental Station 400
200 Powder Mill Road
Wilmington, DE 19803 USA

Supplied by:
OXOID Limited
Thermo Fisher Scientific,
Wade Road,
Basingstoke,
Hampshire, UK,
RG24 8PW

fulfils the requirements of the NordVal Validation Protocol. The performance of the BAX® System Real-Time PCR Assay for *Campylobacter jejuni / coli* and *lari* has been compared against the following 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 results obtained are in accordance with ISO 16140-2:2016 and they document no statistical difference in the performances between the methods for poultry faeces on cloacae swabs for levels above 100 cfu/g.

Date: 1 December 2017

Yours sincerely

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
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 an 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 report 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. 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 for *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.

## COMPARISON STUDY

The comparison study was carried out by the Danish Veterinary and Food Administration, Region North in 2009.

### 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 (≥ 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-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**
The Inclusivity/exclusivity tests performed at the expert laboratory Cherney Microbiological Services, Green Bay, Wisconsin USA for AOAC Research Institute in 2007. 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 35 non-target strains. Thus, 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: 1.0 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.