



Experience from validation of a rapid method for  
Nordval approval.

Qualitative analysis of *Campylobacter jejuni*,  
*coli* and *lari* in chicken cloacae swabs samples  
by PCR (DuPont Qualicon BAX Q7).



# The Danish Veterinary and Food Administration, Region West

- Microbiological laboratory in Aalborg.
- Analysis of samples taken by the Food authorities in the middle and north of Jutland. Control and surveys.
- Analysis of commercial samples.
- Other activities for instance validation, consultancy.
- Laboratory manager: Majbritt Moos.



## The alternative rapid method

- DuPont Qualicon BAX Q7 (Called BAX)
- The BAX detection system is a rapid method for detecting pathogens or other organisms in food and environmental samples.
- Real-time PCR method.
- Nordval-validation for Salmonella (foods, feed and environmental samples) and Campylobacter (Chicken cloacae swabs)



## Why use this rapid method?

- No enrichment (fecal samples - high numbers of campylobacter if present).
- No complicated extraction step.
- Few handling steps.
- Closed system for the PCR (low risk of polluting the surroundings)
- Simple result -can be used immediately.



## Flow of the rapid method

- Sampling of cloacae swabs.
- Suspension in 2 mL 0,9% NaCl / 0,1% peptone kept at 5° C.





## Flow of the rapid method

- 50  $\mu\text{L}$  suspension is mixed with 200 lysis  $\mu\text{L}$  buffer (previously mixed with protease).
- Lysis: 20 min. at 37° C.
- Protease-inactivation: 10 min. at 95° C.
- Cooling to 5° C.





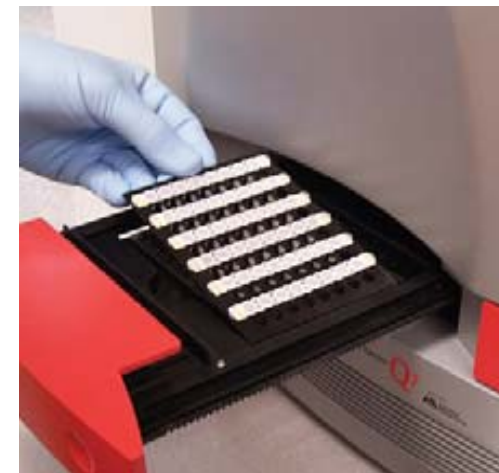
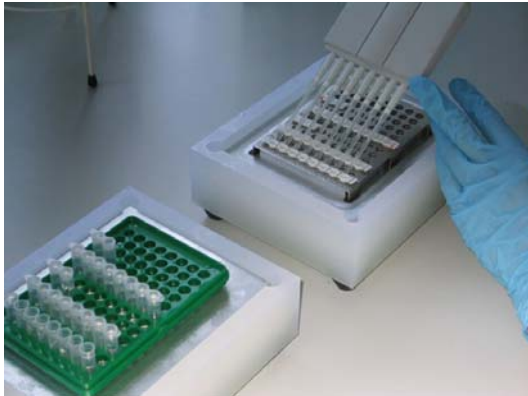
# Flow of the rapid method





## Flow of the rapid method

- 30  $\mu$ L added to PCR tubes containing PCR-mix

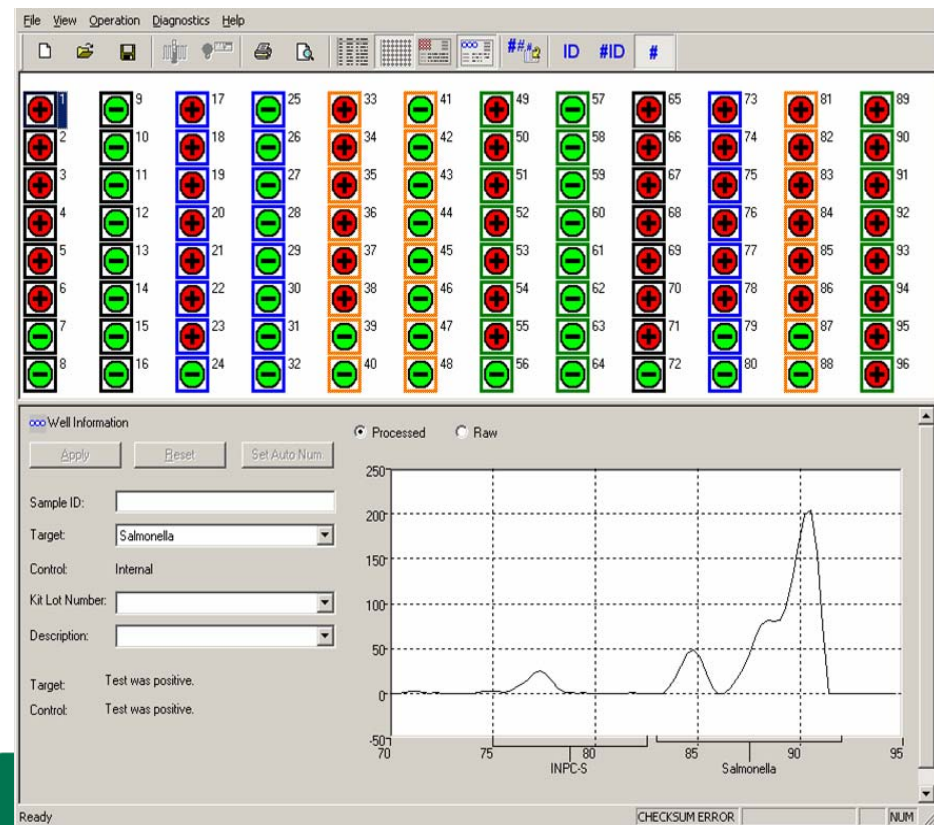


- Real-Time PCR analysis by the BAX Q7 and the BAX Real-Time PCR assay for *Campylobacter coli/jejuni/lari*.



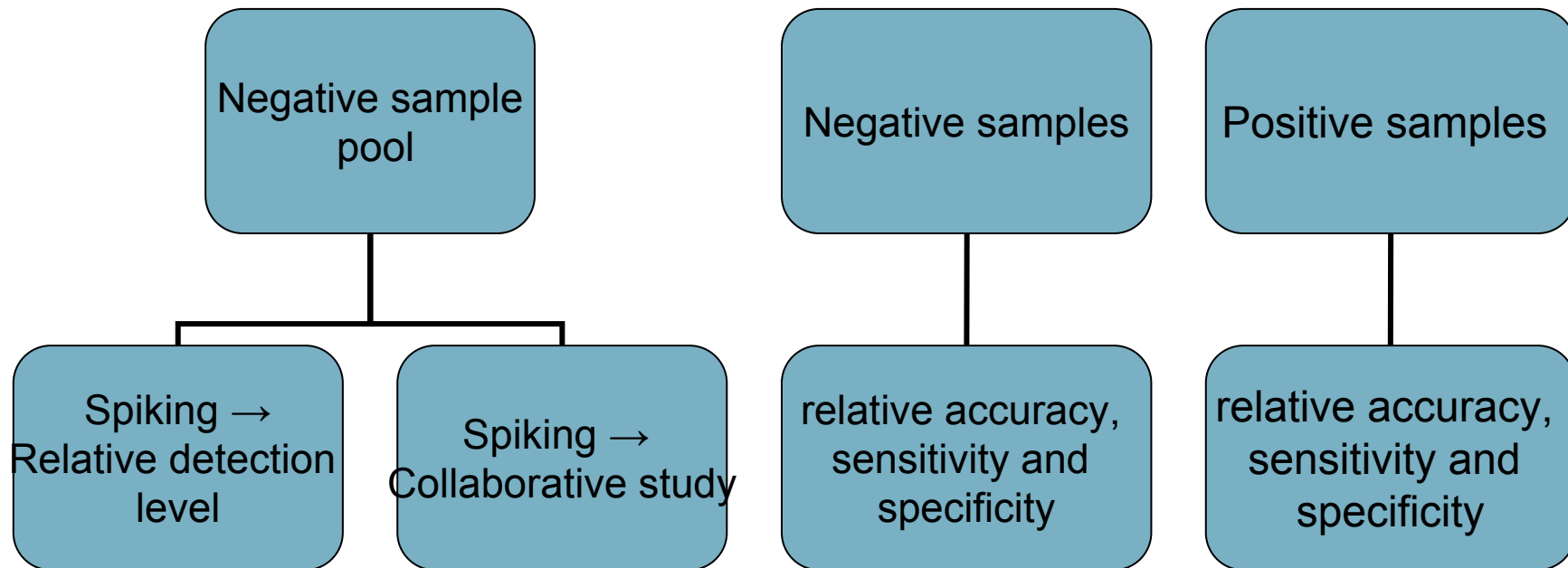
# Flow of the rapid method

- Reading the results.





# Planning the validation



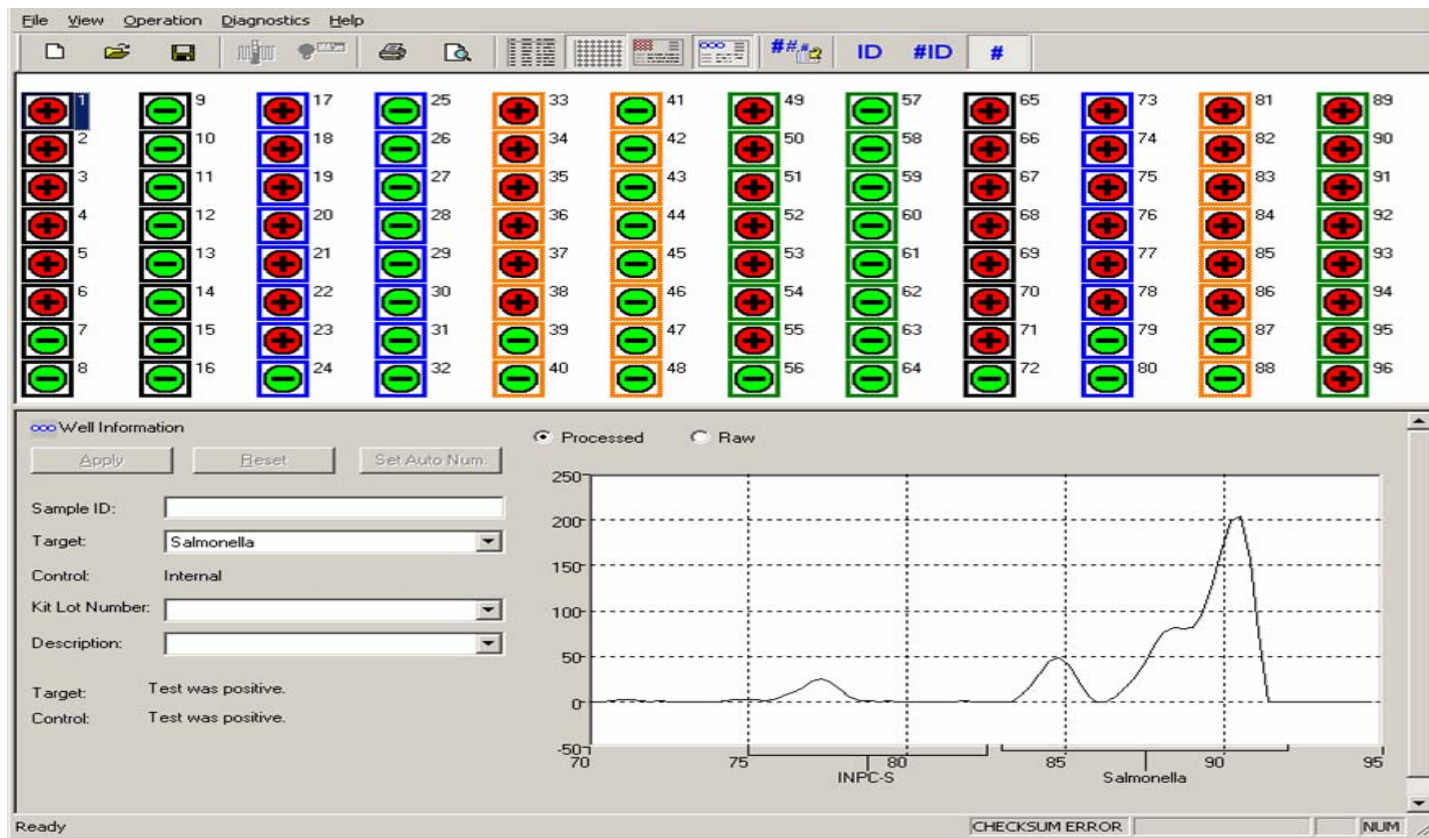


## The validation was carried out

- The analyses were carried out in May and June 2009 by Annie Graugaard and Kristina Pedersen.
- The collaborative study was carried out in June 2009.



# Results





# METHOD COMPARISON STUDY – RELATIVE ACCURACY, SENSITIVITY AND SPECIFICITY

- **Background**
- The purpose of the trial was to demonstrate equivalence between Campylobacter PCR Real Time method on BAX Q7 and the reference method ISO 10272-1 in accordance with the NordVal validation criteria. To determine the test performance (relative accuracy, relative sensitivity and relative specificity) 60 samples were tested to give approximately 30 positive and 30 negative results by the reference method and the alternative method.



# Results: BAX Q7 and the reference method ISO 10272-1

## After screening:

PA (positive agreement) = 30

PD (positive deviation) = 0

ND (negative deviation) = 0

NA (negative agreement) = 30

## After confirmation:

PA (positive agreement) = 30

TP (true positives) = 0

FN (false negatives) = 0

NA (negative agreement) = 30

FP (false positives) = 0



Relative accuracy (AC), Relative sensitivity (SE) and Relative specificity (SP) after confirmation (ISO only).

Matrices	PA	NA	FN	TP	FP	Sum	Relative Accuracy AC (%)	Relative sensitivity SE (%)	Relative specificity SP (%)
						N	$\frac{(PA+NA+FP) \times 100}{N}$	$\frac{(PA+TP) \times 100}{PA+FN}$	$\frac{NA \times 100}{NA+FP}$
TOTAL	30	30	0	0	0	60	100	100	100

$$N = PA + NA + FN + TP + FP$$



## Method Comparison study - Detection Level

- **Background**
- The purpose of this trial was to demonstrate the relative detection level between Campylobacter PCR Real Time method on BAX Q7 and the reference method ISO 10272.
- The volume of sample analysed by the BAX Q7-method was 50  $\mu$ l and for the ISO 10272 1 ml. The difference sample volumes was 20 times. The same detection level when comparing these two methods was therefore not expected.
- It must also be said that the ISO 10272 is developed and validated for the matrixes food and feed.



## Method Comparison study - Detection Level

- As was done for the validation of another Realtime PCR method (Nordval reference 017). The detection level of direct plating of a comparable volume of sample was also determined.
- Direct plating is a widely used qualitative campylobacter method for faeces (golden standard) according to Steen Nordentoft, Veterinærinstituttet, Aarhus.

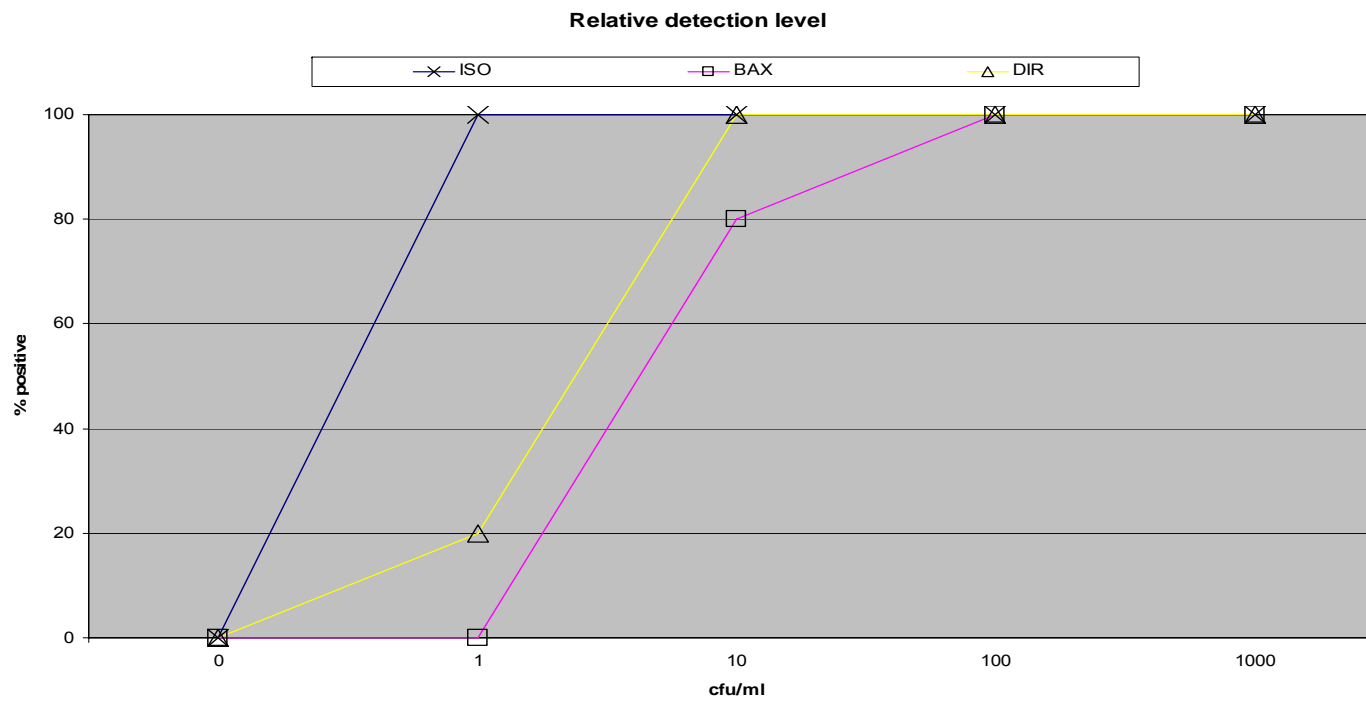


Level	Added bacterial suspension in 100 g negative faeces suspension.	Expected cfu/g
A	None	0-1
B	10 ml C	1-10
C	10 ml D	10-100
D	10 ml E	100-1000
E	0,5 ml of the initial bacterial suspension containing 1-2 mio cfu/mL.	1000-10000



## Results detection level

	0 cfu/ml	1-10 cfu/ml	10-100 cfu/ml	100-1000 cfu/ml	100-1000 cfu/ml
ISO	0 %	100 %	100 %	100 %	100 %
BAX	0 %	0 %	80 %	100 %	100 %
Direct plat.	0 %	20 %	100 %	100 %	100 %





# COLLABORATIVE STUDY

- **Background**
- The aim of the collaborative study was to determine the variability of the results obtained by the alternative method in 7 different laboratories using identical samples.
- The spiked samples for the collaborative study were the same used in the *“Relative detection level”*.



## COLLABORATIVE STUDY

- Duplicates of the spiked samples were prepared for each laboratory to be analysed by the alternative method.
- Lysing procedure: The frozen samples were lysed by the laboratories according to the normal BAX procedure.
- Each laboratory received 10 blinded (coded) cluster tubes for analysing.
- Run by the BAX Q7 Real-Time Campylobacter programme.



Laboratories	0 cfu/ml L <sub>0</sub>	1-10 cfu/ml L <sub>1</sub>	10-100 cfu/ml L <sub>2</sub>	100-1000 cfu/ml L <sub>3</sub>	1000-10000 cfu/ml L <sub>4</sub>
Lab. 1	0/2	0/2	0/2	2/2	2/2
Lab. 2	0/2	0/2	1/2	2/2	2/2
Lab. 3	0/2	0/2	0/2	2/2	2/2
Lab. 4	0/2	0/2	2/2	2/2	2/2
Lab. 5	0/2	0/2	2/2	2/2	2/2
Lab. 6	0/2	0/2	1/2	2/2	2/2
Lab. 7	0/2	0/2	2/2	2/2	2/2
Total	0/14	0/14	8/14	14/14	14/14



## Relative selectivity (Inclusivity/Exclusivity study)

- Background
- The relative selectivity is a measure of
- A the inclusivity: detection of the target microorganism from a wide range of strains, and
- B the exclusivity: the lack of interference from a relevant range of non-target microorganisms.
- This part of the validation was done by DuPont Qualicon.

**Table 1. BAX® system Inclusivity Results for *Campylobacter jejuni/coli/lari***

Strain #	Genus/Species	Source	Result	Strain #	Genus/Species	Source	Result
TD4604	<i>C. coli</i>	Avian	POS	TD6529	<i>C. coli</i>	Avian	POS
TD4631	<i>C. coli</i>	Avian	POS	TD6531	<i>C. coli</i>	Avian	POS
TD4923	<i>C. coli</i>	Avian	POS	TD6539	<i>C. coli</i>	Avian	POS
TD4928	<i>C. jejuni</i>	Avian	POS	TD6540	<i>C. coli</i>	Avian	POS
TD4937	<i>C. jejuni</i>	Avian	POS	TD6551	<i>C. jejuni</i>	Avian	POS
TD4960	<i>C. jejuni</i>	Avian	POS	TD6553	<i>C. jejuni</i>	Avian	POS
TD6295	<i>C. jejuni</i>	Avian	POS	TD6555	<i>C. jejuni</i>	Avian	POS
TD6296	<i>C. jejuni</i>	Avian	POS	TD6557	<i>C. jejuni</i>	Avian	POS
TD6297	<i>C. jejuni</i>	Avian	POS	TD6560	<i>C. jejuni</i>	Avian	POS
TD6300	<i>C. jejuni</i>	Avian	POS	TD6561	<i>C. jejuni</i>	Avian	POS
TD6301	<i>C. jejuni</i>	Avian	POS	TD6562	<i>C. lari</i>	Avian	POS
TD6308	<i>C. coli</i>	Avian	POS	TD6563	<i>C. lari</i>	Avian	POS
TD6311	<i>C. coli</i>	Avian	POS	TD6564	<i>C. lari</i>	Avian	POS
TD6312	<i>C. coli</i>	Avian	POS	TD6566	<i>C. lari</i>	Avian	POS
TD6321	<i>C. coli</i>	Avian	POS	TD6567	<i>C. lari</i>	Avian	POS
TD6423	<i>C. lari</i>	Avian	POS	TD6568	<i>C. lari</i>	Avian	POS
TD6424	<i>C. lari</i>	Avian	POS	TD6569	<i>C. lari</i>	Avian	POS
TD6425	<i>C. lari</i>	Avian	POS	TD6570	<i>C. lari</i>	Avian	POS
TD6481	<i>C. lari</i>	Clinical	POS	TD6571	<i>C. lari</i>	Avian	POS
TD6483	<i>C. lari</i>	Clinical	POS	TD6577	<i>C. lari</i>	Avian	POS
TD6484	<i>C. lari</i>	Clinical	POS	TD6622	<i>C. lari</i>	Avian	POS
TD6485	<i>C. lari</i>	Clinical	POS	TD7012	<i>C. jejuni</i>	Avian	POS
TD6486	<i>C. lari</i>	Clinical	POS	TD7018	<i>C. jejuni</i>	Avian	POS
TD6525	<i>C. coli</i>	Avian	POS	TD7019	<i>C. jejuni</i>	Avian	POS
TD6526	<i>C. coli</i>	Avian	POS	TD7023	<i>C. coli</i>	Avian	POS
TD6527	<i>C. coli</i>	Avian	POS	TD7026	<i>C. jejuni</i>	Avian	POS


**Table 2 BAX® system Exclusivity Results for *Campylobacter jejuni/coli/lari***

Strain #	Genus/Species	Source	Result	Strain #	Genus/Species	Source	Result
DD 2901	<i>Bacillus cereus</i>	Cream cake	NEG	TD 6537	<i>Campylobacter fetus venerealis</i>	Unknown	NEG
ATCC 25408	<i>Citrobacter diversus</i>	Human clinical	NEG	ATCC BAA-1059	<i>Campylobacter upsaliensis</i>	Human	NEG
ATCC 33379	<i>Edwardsiella hoshinae</i>	Avian	NEG	ATCC 33562	<i>Campylobacter sputorum</i>	Bovine	NEG
DD 10549	<i>Enterococcus cecorum</i>	Avian	NEG	ATCC 51210	<i>Campylobacter helveticus</i>	Feline	NEG
ATCC 35038	<i>Enterococcus gallinarum</i>	Avian	NEG	ATCC 43264	<i>Campylobacter mucosalis</i>	Porcine	NEG
DD 10674	<i>Enterococcus saccharolyticus</i>	Straw bedding - Avian	NEG	DD 6832	<i>Shigella sonnei</i>	Unknown	NEG
DD 1722	<i>Escherichia coli</i> O127:HNM	PSU Reference Laboratory	NEG	ATCC 43952	<i>Staphylococcus arlettae</i>	Avian	NEG
ATCC 33821	<i>Escherichia vulnaris</i>	Human clinical	NEG	ATCC 35539	<i>Staphylococcus gallinarum</i>	Avian	NEG
DD 6523	<i>Klebsiella oxytoca</i>	Ground beef	NEG	ATCC 9610	<i>Yersinia enterocolitica</i>	Human clinical	NEG
ATCC 33403	<i>Kurthia zopfii</i>	Avian	NEG	DD 2992	<i>Salmonella ser. Lille</i>	Unknown	NEG
ATCC 19111	<i>Listeria monocytogenes</i>	Avian	NEG	DD 1261	<i>Salmonella ser. Newport</i>	Avian	NEG
DD 3064	<i>Morganella morganii</i>	Environmental swab	NEG	ATCC 49616	<i>Arcobacter butzleri</i>	Human clinical	NEG
DD 6121	<i>Proteus mirabilis</i>	Avian	NEG	TD 6513	<i>Arcobacter butzleri</i>	Unknown	NEG
ATCC 27853	<i>Pseudomonas aeruginosa</i>	Human clinical	NEG	TD 7030	<i>Arcobacter cryaerophilus</i>	Unknown	NEG
ATCC 43972	<i>Salmonella enterica salame</i>	Unknown	NEG	TD 7011	<i>Campylobacter fetus fetus</i>	Unknown	NEG
DD 1550	<i>Salmonella ser. Abaetetuba</i>	Unknown	NEG	TD 7013	<i>Campylobacter fetus fetus</i>	Unknown	NEG
DD 3017	<i>Salmonella ser. Dublin</i>	Unknown	NEG	ATCC 13076	<i>Salmonella ser. Enteritidis</i>	Unknown	NEG
TD 6536	<i>Campylobacter fetus venerealis</i>	Unknown	NEG	DD 626	<i>Lactobacillus viridescens</i>	Cured meat	NEG
DD 659	<i>Lactococcus lactis</i>	Unknown	NEG	DD 687	<i>Lactobacillus carnis</i>	Vacuum pack lamb	NEG



- ***Inclusivity***
- All of the 52 Campylobacter strains (18 C. jejuni, 15 C. coli and 19 C. lari) tested were positive.
- ***Exclusivity***
- All of the 38 non-target strains tested negative, including 8 Campylobacter non-jejuni/coli/lari.



## Conclusion

- Results from the comparative study, after screening, and after confirmation shows relative accuracy, sensitivity and specificity of 100 % all. This shows that the Bax Q7 method was as accurate, specific and sensitive as ISO 10272, when the natural positive and negative samples were analysed.
- The collaborative study results in a specificity (sp) of 100 % for all three methods (ISO 10272, direct plating and BAX) for the 7 laboratories and the expert laboratory.



## Conclusion

- The Accuracy and the sensitivity are different depending on spiking levels and the reference method used.
- Accuracy and sensitivity of 100 % is achieved for all three methods and for both the expert laboratory and the 7 laboratories in the collaborative study, when analysing samples containing 100-1000 cfu/ml (level L3) and above.
- The level of *Campylobacter* in the 30 natural positive samples of chicken faeces must therefore have been at least 100 cfu/ml.
- 100 cfu/ml is the detection level for the BAX Q7 method.



## How this rapid method is used now

- Survey of the campylobacter content in broiler flocks (1 swab sample representing 25 birds)
- Settlement of the broiler producers. (Campylobacter free are paid a little more)
- Initially: used for the labelling: "campylobacter free" (20 swabs samples per broiler flock, each swab representing 25 birds)
- Advantage: Not educated labour can perform the analysis with the assistance of a lab. technician.







Thank you for your attention.

## NordVal Certificate

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

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