

**ThermoFisher**  
S C I E N T I F I C

*The world leader in serving science*

# Detection of Food-Borne Pathogens with the BAX<sup>®</sup>-PCR

# Structure

- The BAX<sup>®</sup>-System
- Detection of *Salmonella spp.* from spices and animal feed
- Multiplex-Real-Time-PCR for the detection of *Campylobacter jejuni*, *C. coli* und *C. lari*

# The BAX<sup>®</sup> system: Scope of delivery

- Thermocycler
- Computer with printer
- BAX -Software
- 2 heating blocks
- 2 cooling blocks
- 2 adjustable pipettes
- Dispenser
- 8 channel pipette
- 2 Capping/decapping tools
- Consumables for 192 assays



# BAX<sup>®</sup> kits

## Kits available:

- ✓ *Salmonella*
- ✓ *Listeria monocytogenes*
- ✓ Genus *Listeria*
- ✓ *Enterobacter sakazakii*
- ✓ *E. coli* O157:H7
- ✓ Yeast and molds
- ✓ Real-time *Staphylococcus aureus*
- ✓ Real-time *Campylobacter jejuni/coli/lari*
- ✓ 8 h *Listeria sp.* (RT-PCR for environmental samples)



Each kit contains 96 assays:

- PCR tubes containing all reagents
- Protease and lysis buffer

# Food types

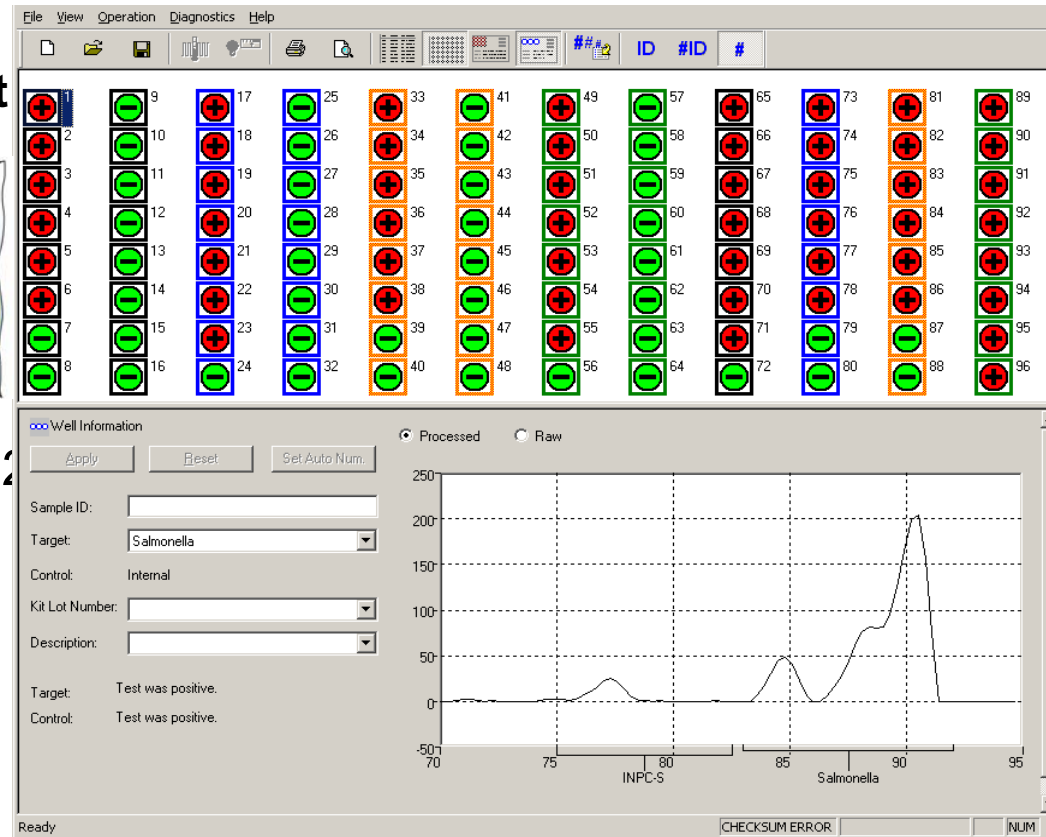
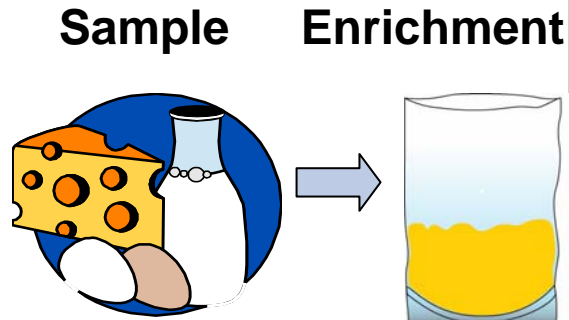
## Food

- Meat/Fish
- Chocolate
- Flavours
- Milk and milk products
- Spices
- Convenient food
- Cereals
- Etc. etc.....

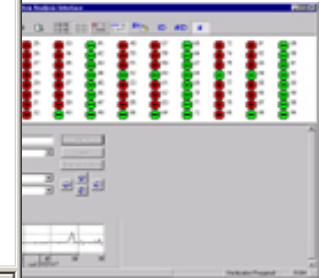
## Other samples

- Environmental samples
- Feed

# BAX<sup>®</sup> protocol (enrichment + PCR)







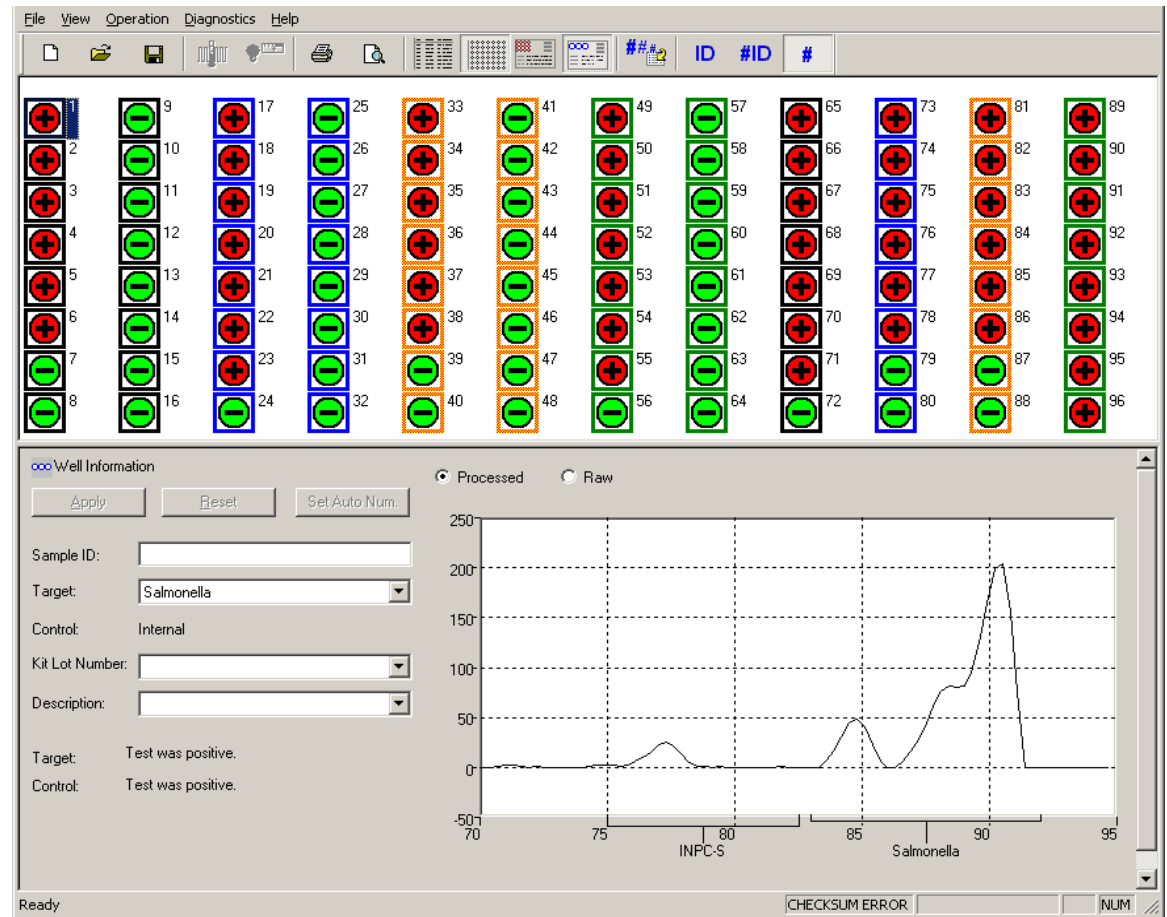
etection +  
analysis



- Completely automated detection and analysis
- Simultaneous analysis *Salmonella*, *E. coli*, *E. sakazaki*, *E. monocytogenes*, *L. sp.*

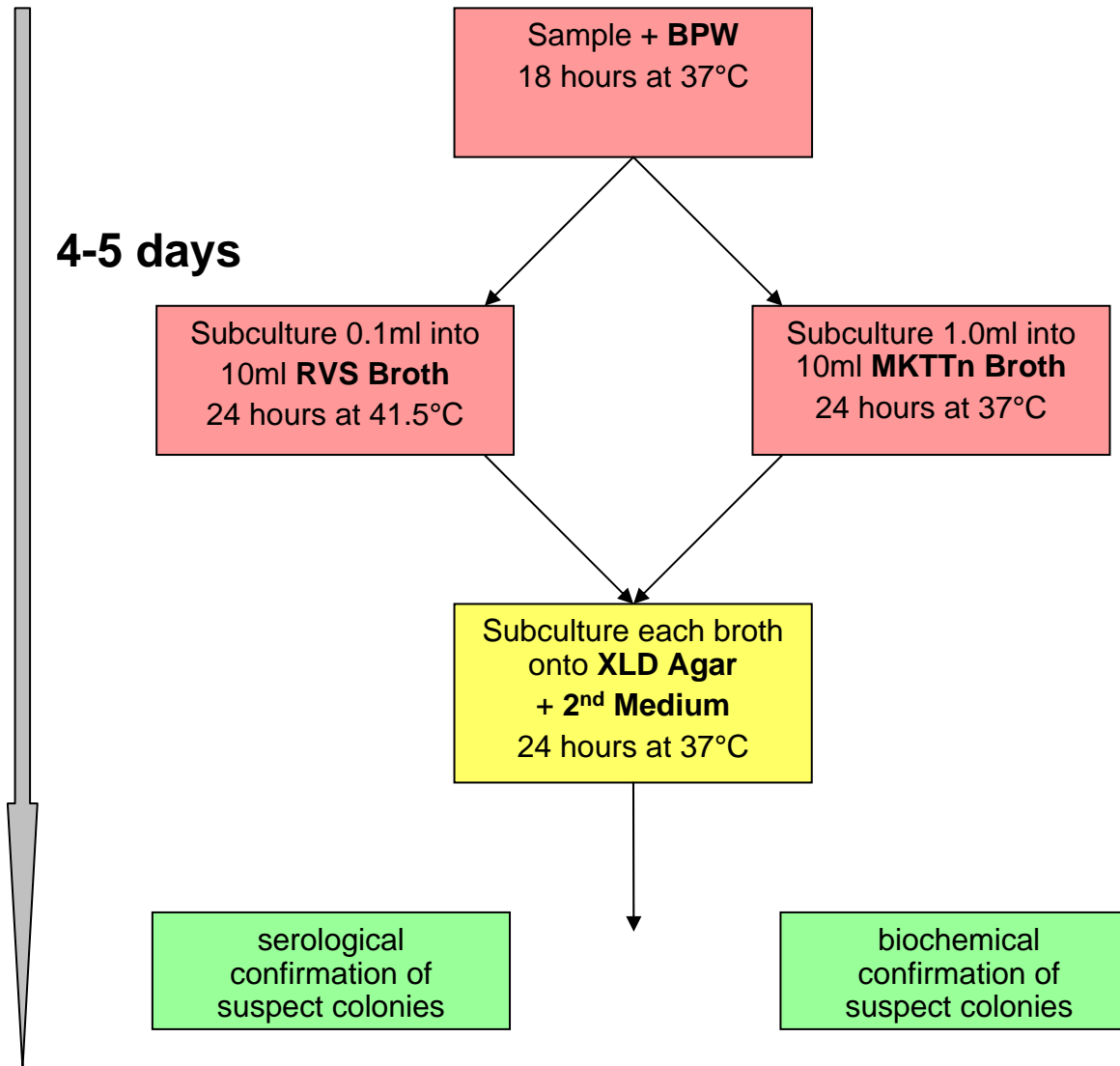
# Result Screen

-  Negative
-  Positive
-  Indeterminate result
-  Signal error



*Salmonella, E. coli 0157:H7 (MP), Enterobacter sakazakii, Genus Listeria and L. monocytogenes can be analyzed in parallel*

# Salmonella detection (ISO 6579:2002)



# BAX protocol for the detection of Salmonella; meat samples

enrichment

sample in BPW (1:10)  
Incubation: 18±2 h bei 37°C

BAX®-PCR

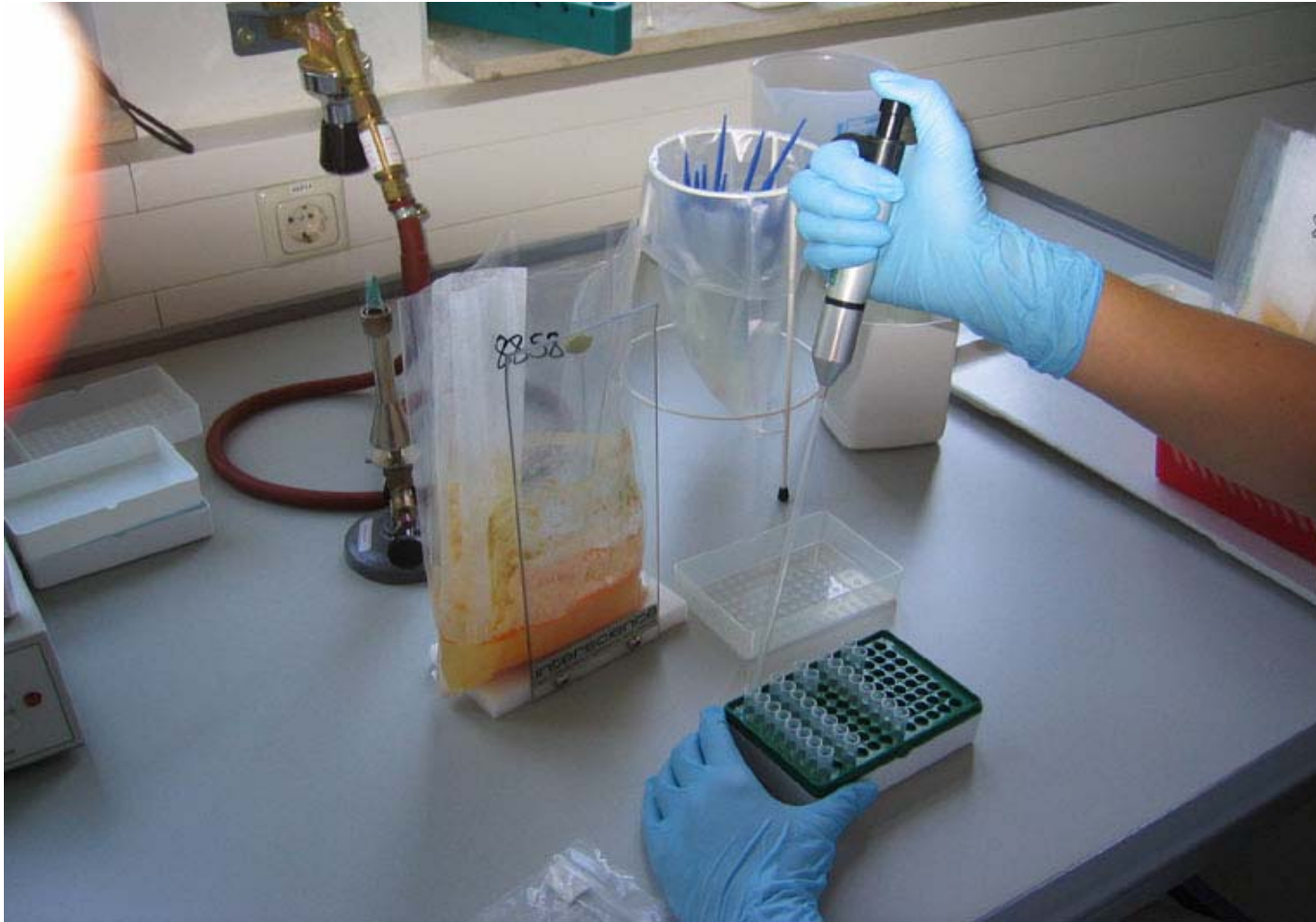


Result after  
~ 23 Stunden

# Enrichment



# Transfer of enrichment broth into empty cluster tubes



Transfer (5  $\mu$ l) in 200  $\mu$ l lysis buffer

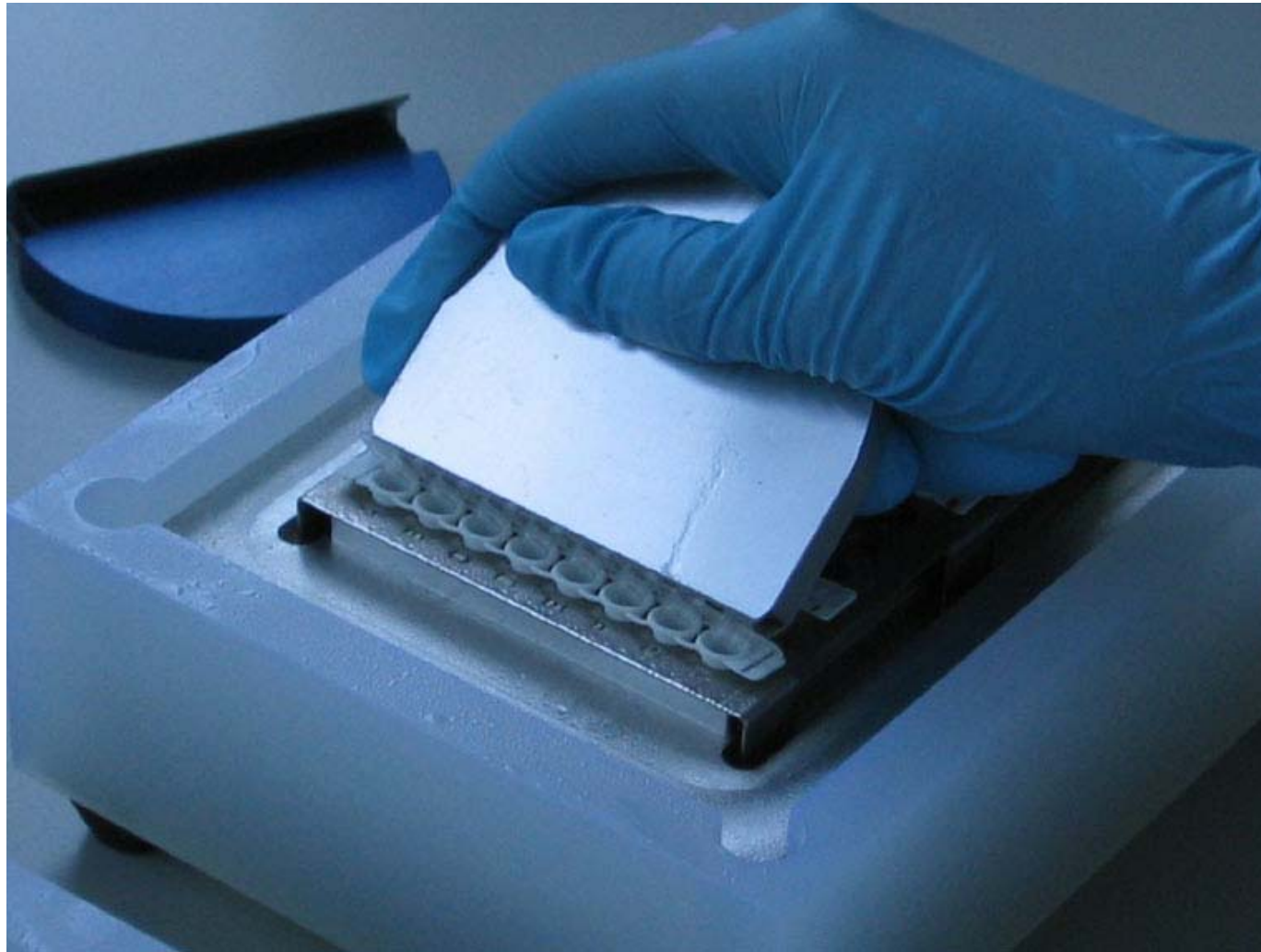


# Enzymatic cell lysis in heating blocks: 37°C/55 °C and 95°C

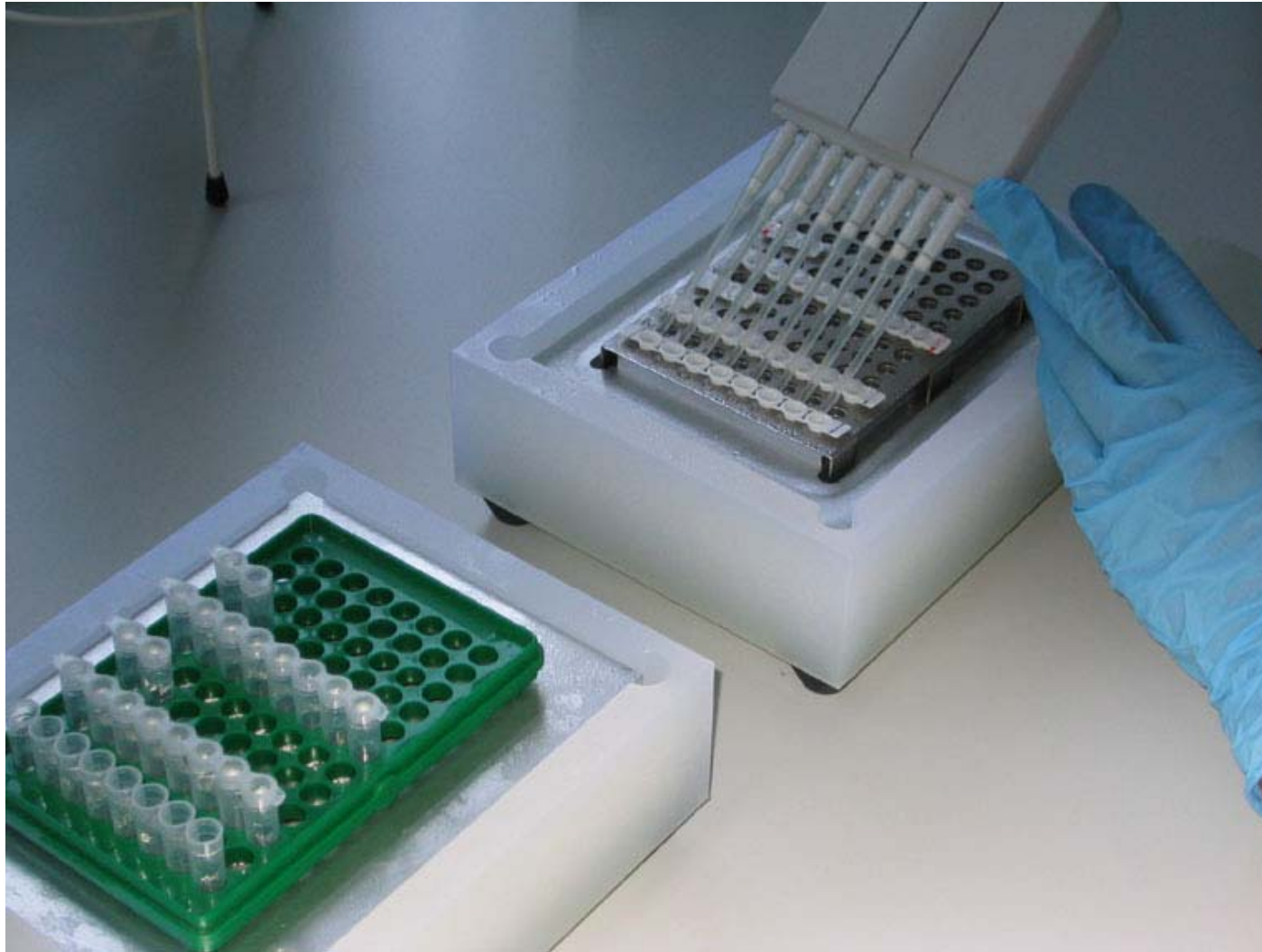


- 20 min, 37°C (Listerien 60 min, 55°C)
- Protein removal 10 min 95°C (all kits)
- > 5 min cooling

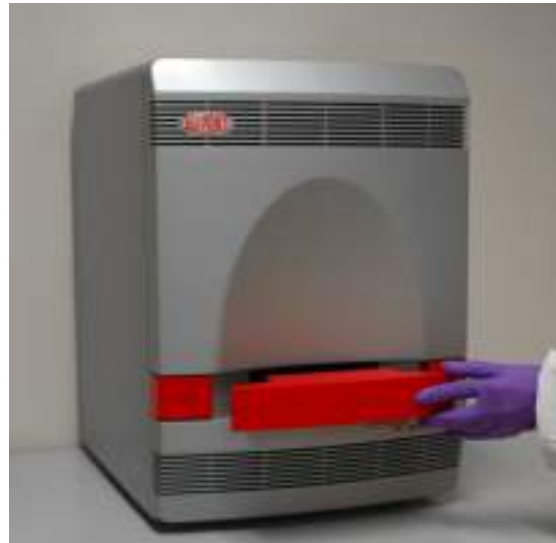
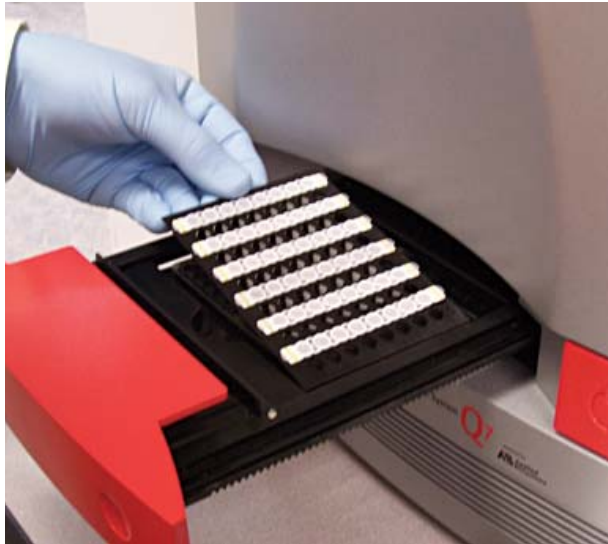
# Opening of PCR tubes



# Transfer of 50 $\mu$ l lysate into the PCR tubes



# Preparing a sample table, starting the PCR



# International certificates



## AOAC International Official Method

*Salmonella* #2003.09; *L. Monocytogenes* #2003.12



## AOAC-RI Performance Tested Method

*Salmonella* #100201; *Listeria monocytogenes* #070202; *E. Coli* O157:H7 #010401, *Campylobacter real-time PCR* #040702, *S. aureus* #120701



## USDA-FSIS Adoption

*Salmonella* #MLG 4C.01; *Listeria monocytogenes* #MLG 8A.00



## Health Canada Certification

*Salmonella* #MFLP-29; *Listeria monocytogenes* #MFLP-28  
*E. coli* O157:H7 #MFLP-30; *Enterobacter sakazakii* #MFLP-27



## AFNOR EN-ISO 16140 Certification

*Salmonella* #QUA [18/3-10/06](#)



## NordVal Certification

*Salmonella* #2003-20-5408



## Brazil MAPA Official Reference Method

*Salmonella* #41



## Japanese Ministry of Health, Labour and Welfare

*Listeria*; *Listeria monocytogenes*

# NordVal Certificate



NordVal / NMKL  
c/o National Veterinary Institute  
PB 8156 Dep, 0033 Oslo, Norway  
www.nmkl.org



## NordVal Certificate

Issued for:	BAX Salmonella PCR (BAX Classic and BAX Q7) BAX System with Automated Detection PCR Assay for Screening Salmonella
NordVal No:	030
First approval date:	20 November 2003
Renewal date:	1 June 2007
Valid until:	1 June 2009

Oslo, 19 June 2007

OXOID A/S Thermo Fisher Scientific Lunikvej 28, DK-2670 Greve, Denmark has applied for NordVal validation of BAX System with Automated Detection PCR Assay for Screening Salmonella in food, feed and environmental samples. The producer of the product is Dupont Qualicon, Bedford Bldg, 3531 Silverside Road, Wilmington, DE 19810 USA.

The method is validated against ISO 6579:2002. BAX is also an AOAC method 2003.09. NordVal has studied the enclosures to the application and evaluated the results obtained in the validations by AFNOR and AOAC Research Institute. ISO 16140 was used for the AFNOR validation. NordVal has concluded that it has been satisfactorily demonstrated that the requirements of the NordVal validation protocol are fulfilled for foods, feeds and environmental samples. The limit of detection for the method is demonstrated to be 2-7 cfu/25 g or ml. The BAX Salmonella PCR System (BAX Classic and BAX Q7) can be used without further confirmation.

In the letter to the applicant it is stated, that NordVal should be informed of any changes of the method, and that every two years an application for renewal of the certificate should be forwarded to NordVal. For the renewal of the NordVal certificate the method must be validated against the latest version of the reference method.

Yours sincerely

  
Sven Qvist  
Chair of NordVal

  
Hilde Skår Nori  
NMKL Secretary General

NMKL /NordVal Tel: +47 23216249 / +47 46 888807 E-mail: nmkl@vetinst.no

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# BAX system for the detection of salmonella in spices

Alexandra Ehrig  
Institut Dr. Appelt, Leipzig



Anis (anise)	Gemüsebrühe	Paprika (paprika)	Sellerie
Arzneifenchel	Ingwer	Paprika Rosen	Senf
Bärlauch	Karottengranulat	Petersilie	Spargelpulver
Basilikum	Karottenpulver	Pfeffer grün	Speckstippe
Bochshornklee	Knoblauch (garlic)	Pfeffer schwarz	Thymian (thyme)
Bohnenkraut	Koriander	Pfeffer weiss	Toastzwiebelpulver
Brennesselblatt	Kräutermischungen	Pilzgewürzsalz	Tomatenflocken
Cayenpfeffer	Kurkuma	Pilzpulver	Vata Tee
Chili	Lauch	Piment	Zimt
Chinabratengewürz	Lebkuchengewürz	Pitta Tee	Zitronengrundstoff
Curcuma	Lorbeerblätter	Reismehl	Zitronenöl
Curry	Majoran	Röstzwiebel	Zwiebel (onion)
Dill	Muskatnuss	Rosmarin (rosemary)	Zwiebelgranulat
Entwässerungstee	Nelken (cloves)	Salatkräuter	Zwiebelpulver
Fischgewürz	Oregano (oregano)	Salz	
Gelbsenfsaat	Panade	Schnittlauch	div. Gewürzmischungen

# BAX system for the detection of salmonella in spices

Alexandra Ehrig  
Institut Dr. Appelt, Leipzig



number of samples:	690
spiked samples:	235
accuracy:	
culture- ISO:	94,5%
BAX™ PCR:	99,4%

no PCR inhibition

# Detection of Salmonella from spices and animal feed with the BAX-PCR

Archiv für Lebensmittelhygiene 58, Heft 9/10 (2007), xxx-xxx

58,

Gesellschaft für Umweltanalytik mbH, Osnabrück

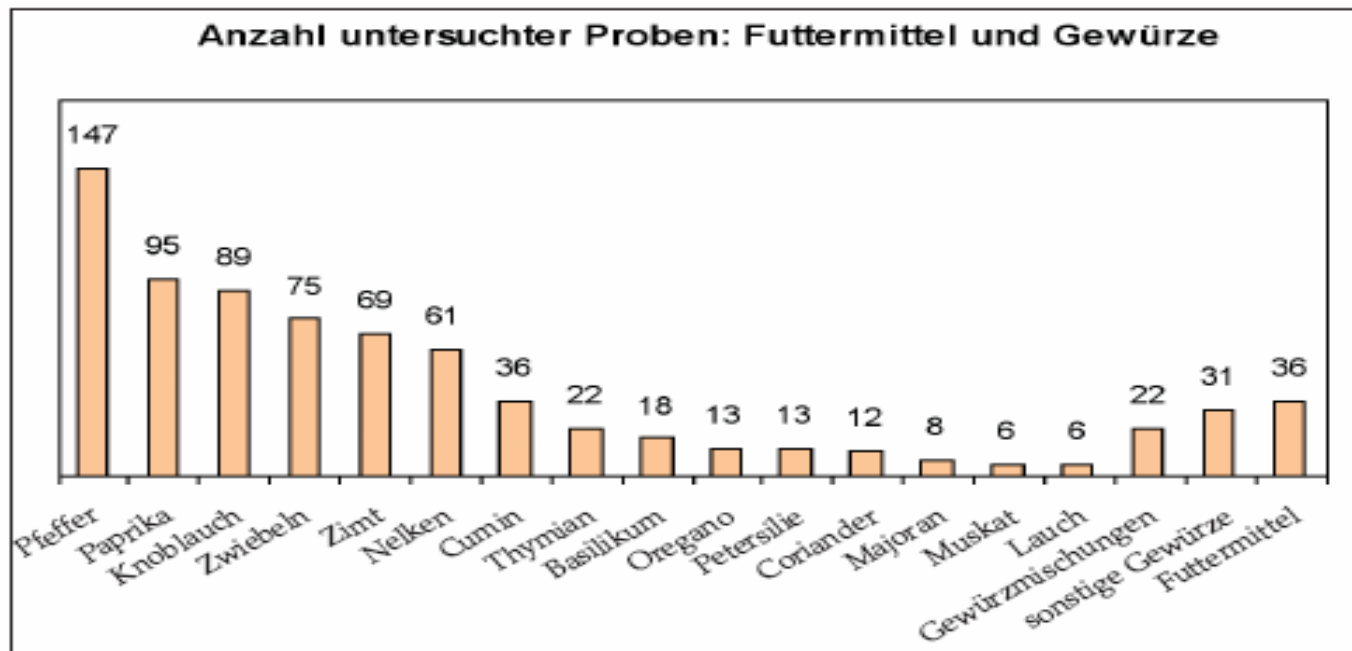
## **Nachweis von Salmonellen in Gewürzen und Futtermitteln. Vergleich des kulturellen Referenzverfahrens nach § 64 LFGB und der BAX<sup>®</sup>-PCR<sup>1</sup> – Validierung eines alternativen Untersuchungsverfahrens**

*Detection of Salmonella from spices and animal feed.  
Comparison of the ISO reference method with the  
BAX-PCR<sup>1</sup> – In-house validation of an alternative method*

210 x 297 mm

# Nachweis von Salmonellen: Futtermittel und Gewürzproben

**ABBILDUNG 1:** *Futtermittel und Gewürzproben der Validierungsstudie*



# Animal feed: Results

Sample	feeding stuff	Salmonella	CFU/25g	PCR	RF
1	Soy pass	no	0	neg	neg
2	Soy pass	S. Yoruba	18	pos	pos
3	Soy pass	S. Yoruba	18	pos	pos
4	Soy pass	S. Yoruba	180	pos	pos
5	Soy pass	S. Yoruba	180	pos	pos
6	Soy pass	S. Mbandaka	12	neg	neg
7	Soy pass	S. Mbandaka	12	neg	neg
8	Soy pass	S. Mbandaka	120	pos	pos
9	Soy pass	S. Mbandaka	120	pos	pos
10	Palm Expeller	no	0	neg	neg
11	Palm Expeller	S. Mbandaka	12	neg	neg
12	Palm Expeller	S. Mbandaka	12	neg	neg
13	Palm Expeller	S. Mbandaka	120	neg	neg
14	Palm Expeller	S. Mbandaka	120	pos	neg
15	Palm Expeller	S. Yoruba	18	neg	neg
16	Palm Expeller	S. Yoruba	18	neg	neg
17	Palm Expeller	S. Yoruba	180	neg	neg
18	Palm Expeller	S. Yoruba	180	neg	neg

Sample	feeding stuff	Salmonella	CFU/25g	PCR	RF
20	Rape flour	S. Yoruba	18	pos	pos
21	Rape flour	S. Yoruba	18	pos	pos
22	Rape flour	S. Yoruba	180	pos	pos
23	Rape flour	S. Yoruba	180	pos	pos
24	Rape flour	S. Mbandaka	180	pos	pos
25	Rape flour	S. Mbandaka	180	pos	pos
26	Rape flour	S. Mbandaka	120	pos	pos
27	Rape flour	S. Mbandaka	120	pos	pos
28	Soy flour	no	0	neg	neg
29	Soy flour	S. Mbandaka	12	pos	pos
30	Soy flour	S. Mbandaka	12	pos	pos
31	Soy flour	S. Mbandaka	120	pos	pos
32	Soy flour	S. Mbandaka	120	pos	pos
33	Soy flour	S. Mbandaka	18	pos	pos
34	Soy flour	S. Yoruba	18	pos	pos
35	Soy flour	S. Yoruba	180	pos	pos
36	Soy flour	S. Yoruba	180	pos	pos

# Detection of Salmonella from spices and animal feed with the BAX-PCR: In- house validation of an alternative method

## Summery of the results

Number of samples: 759

NA: 727

PA: 29

PD: 2

ND: 1

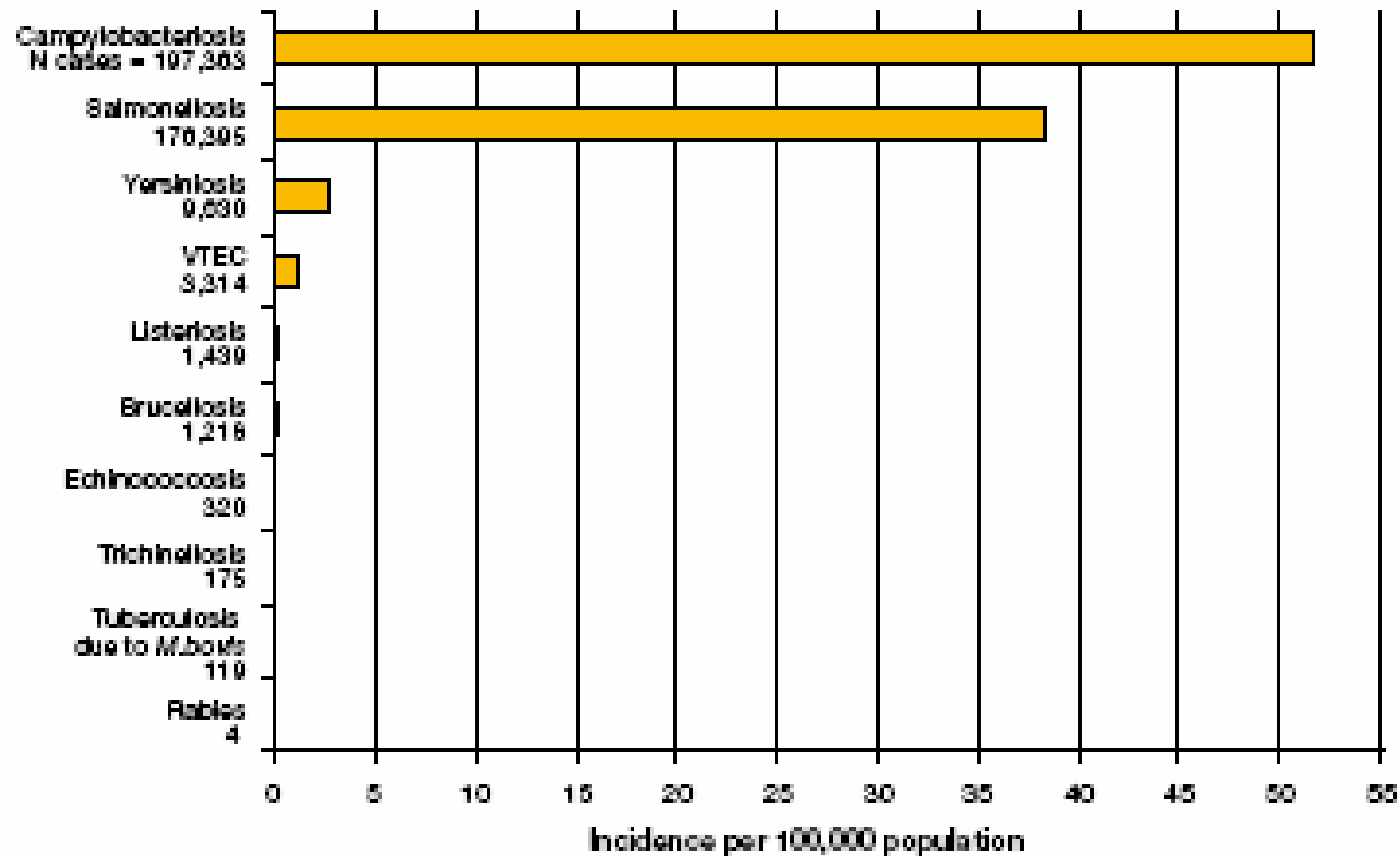
Accuracy BAX-PCR: 99,6%

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  - Monitoring for *Campylobacter* in broiler flocks: preliminary tests to demonstrate the appropriateness of the BAX-System

# Campylobacteriosis

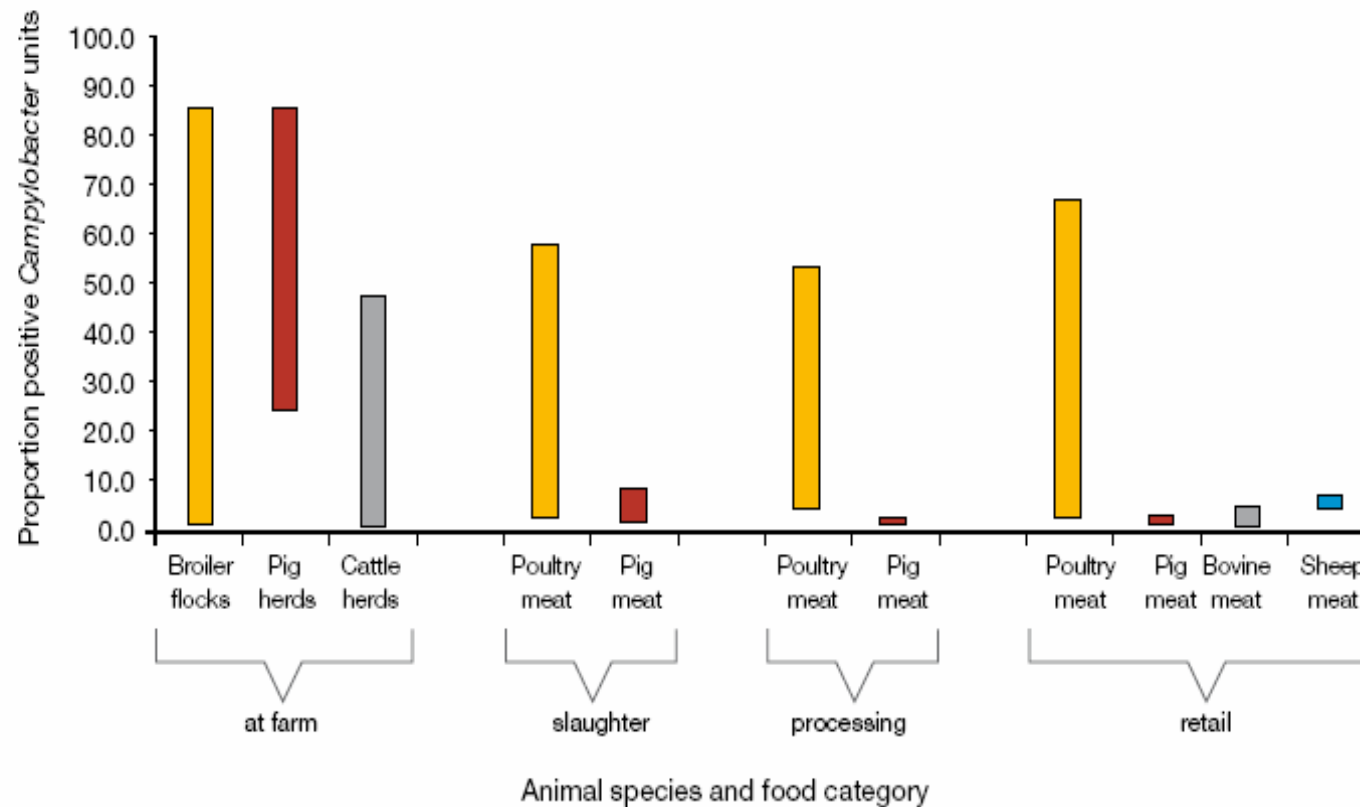
Figure SU1. The reported incidences of the zoonoses in humans, 2005



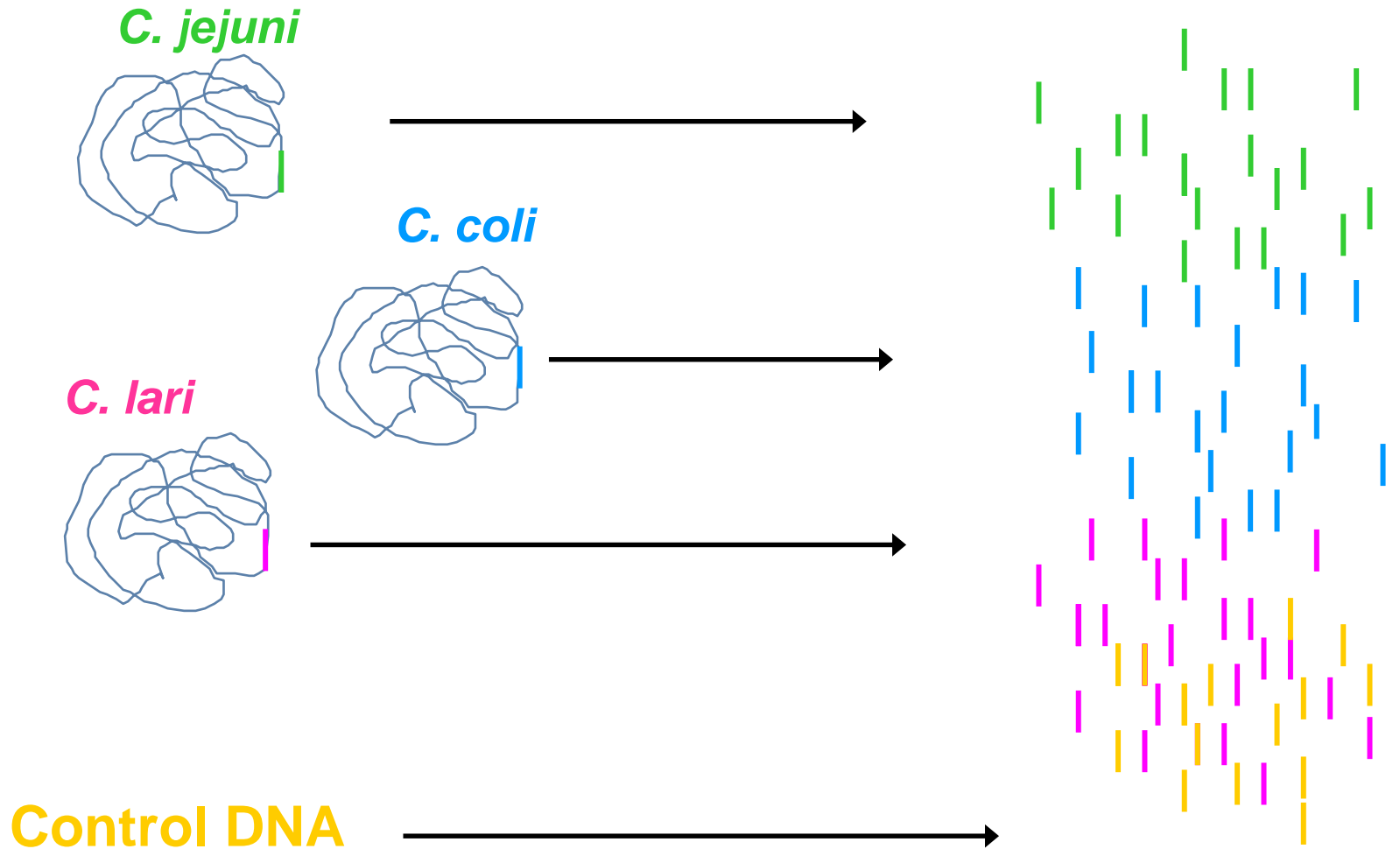
EFSA Journal (2006)

# Campylobacter in pigs, and Poultry; from farm to retail

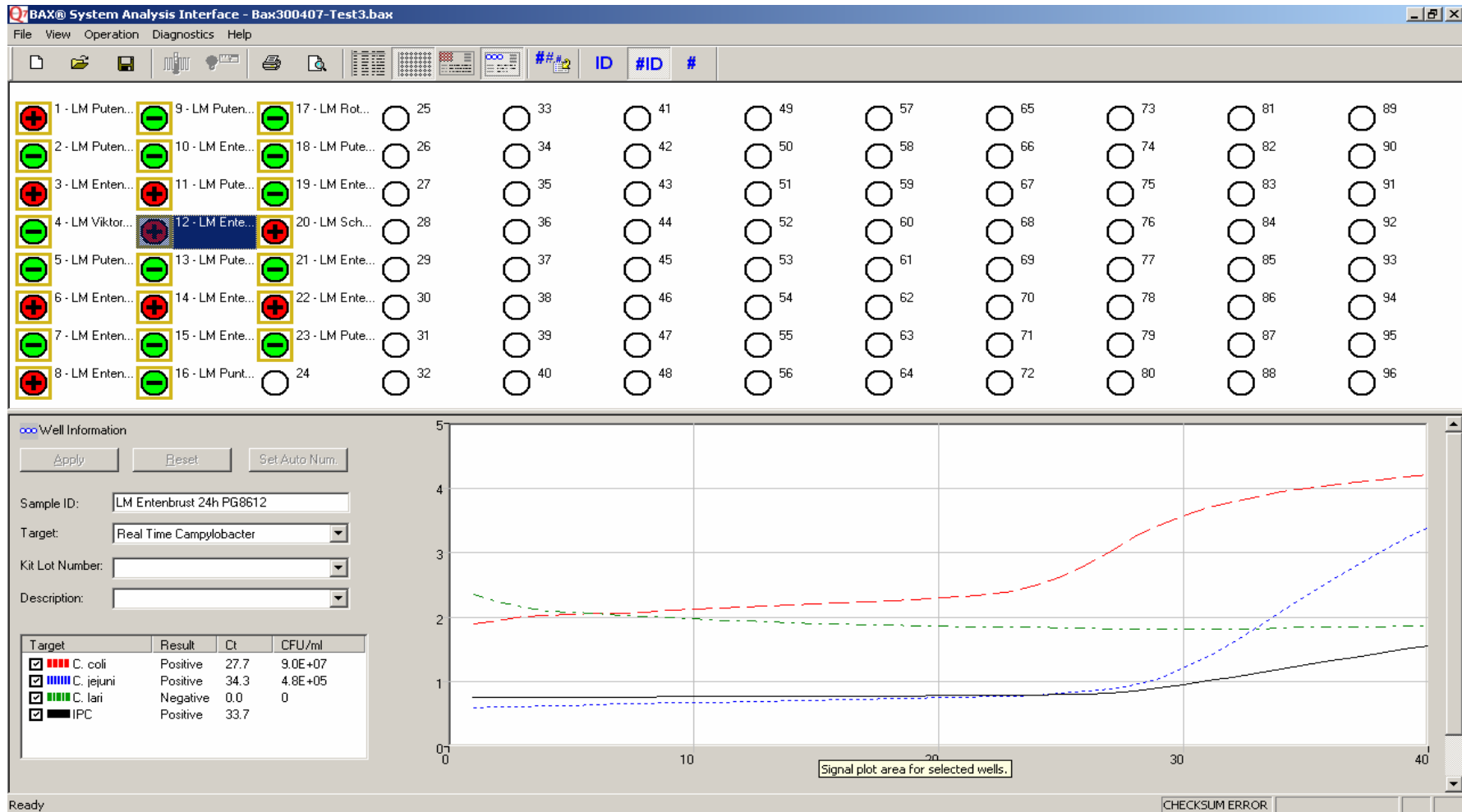
Figure CA2. Minimum to maximum proportions of Campylobacter positive samples reported by MS, by animal species and foodstuff category<sup>1</sup>, 2005



Multiplex-PCR (=PCR with > 1 set of primers)  
4 Taqman probes, dyes VIC, FAM, CY5, NED



# Result screen



# Result screen

BAX® System Analysis Interface - Bax300407-Test3.bax

File View Operation Diagnostics Help

The main interface displays a grid of wells (1-96) with status indicators (red/green circles with plus/minus signs). Well 12 is highlighted in blue. Below the grid, the 'Well Information' panel shows details for well 12, including sample ID, target, and a list of targets with their results and Ct values. A graph on the right shows fluorescence intensity over time for the selected well.

**Well Information**

Apply Reset Set Auto Num.

Sample ID: LM Entenbrust 24h PG8612

Target: Real Time Campylobacter

Kit Lot Number:

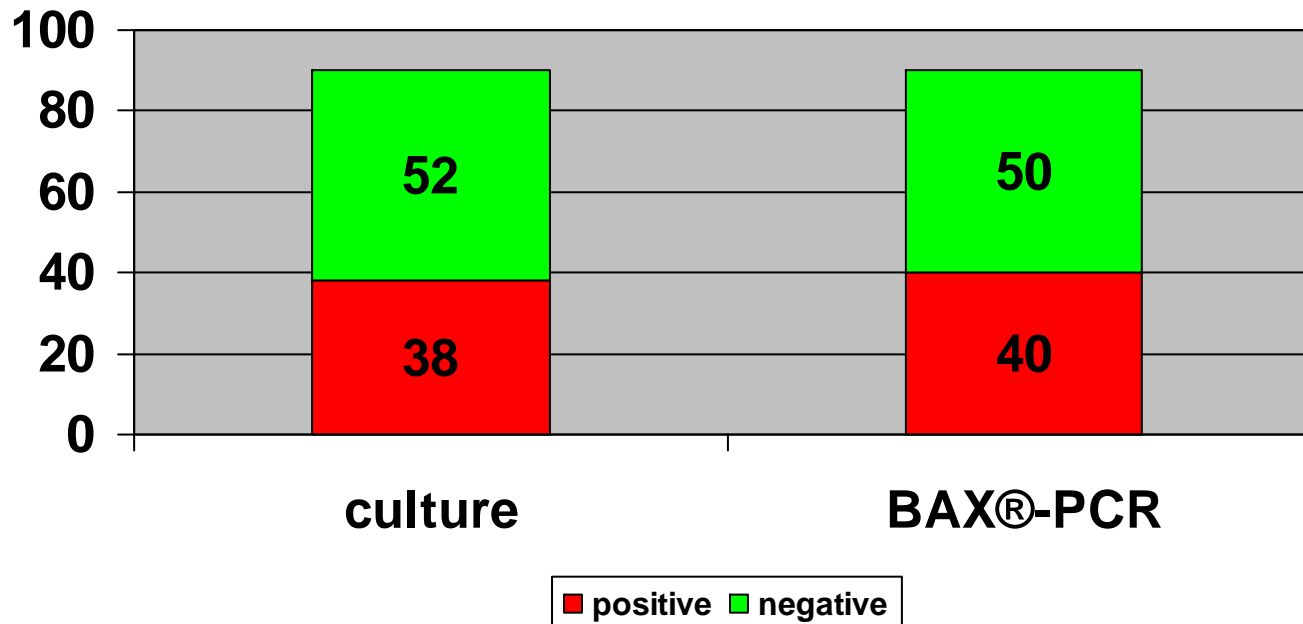
Description:

Target	Result	Ct	CFU/ml
<input checked="" type="checkbox"/> C. coli	Positive	27.7	9.0E+07
<input checked="" type="checkbox"/> C. jejuni	Positive	34.3	4.8E+05
<input checked="" type="checkbox"/> C. lari	Negative	0.0	0
<input checked="" type="checkbox"/> IPC	Positive	33.7	

Ready

Detection of thermophilic *Campylobacter* spp. from food  
LGL Oberschleißheim (Bavaria office for Consumer Protection and Food Safety)

Detection of *Campylobacter* from 90 Food samples  
(65 poultry samples included)  
A comparison of the BAX-PCR with cultural detection  
methods



# International certificates



## AOAC International Official Method

*Salmonella* #2003.09; *L. Monocytogenes* #2003.12

## Campylobacter real-time PCR #040702

## USDA-FSIS Adoption

*Salmonella* #MLG 4C.01; *Listeria monocytogenes* #MLG 8A.00

## Health Canada Certification

*Salmonella* #MFLP-29; *Listeria monocytogenes* #MFLP-28  
*E. coli* O157:H7 #MFLP-30; *Enterobacter sakazakii* #MFLP-27

## AFNOR EN-ISO 16140 Certification

*Salmonella* #QUA [18/3-10/06](#)

## NordVal Certification

*Salmonella* #2003-20-5408

## Brazil MAPA Official Reference Method

*Salmonella* #41

## Japanese Ministry of Health, Labour and Welfare

*Listeria*; *Listeria monocytogenes*

# Inclusivity / Exclusivity

- Inclusivity – 100%
  - 20 *C. jejuni*
  - 15 *C. coli*
  - 19 *C. lari*
- Exclusivity – 100%
  - 6 *Campylobacter* (non- *C. jejuni*, *C. coli*, *C.lari*)
  - 26 non- *Campylobacter*

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  - Monitoring for *Campylobacter* in broiler flocks: preliminary tests to demonstrate the appropriateness of the BAX-System

## Scope of the trial

- 4 control flocks
  - 4 flocks with improved hygienic conditions
- 
- Desinfection of shoes
  - Staff hygiene and dress code
  - Insect traps
  - Pest control

# Detection methods

- BAX-PCR after enrichment in Bolton
- BAX-PCR without enrichment (Direct-PCR)
- Direct culture on selective medium (mCCDA and Karmali)

The Direct-PCR allows the detection of *Campylobacter* within  
~2 hours.



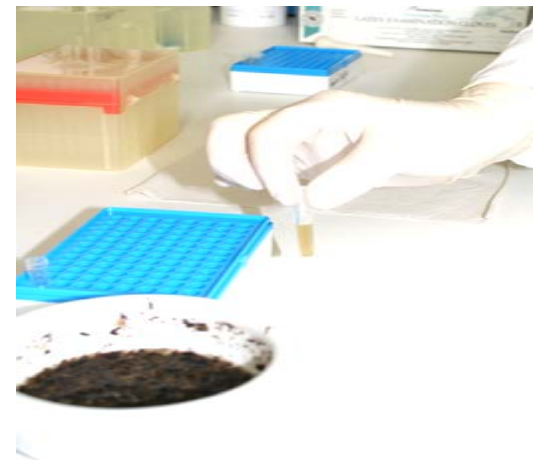
# Sample preparation: Caecal samples



# Sample preparation: Caecal samples



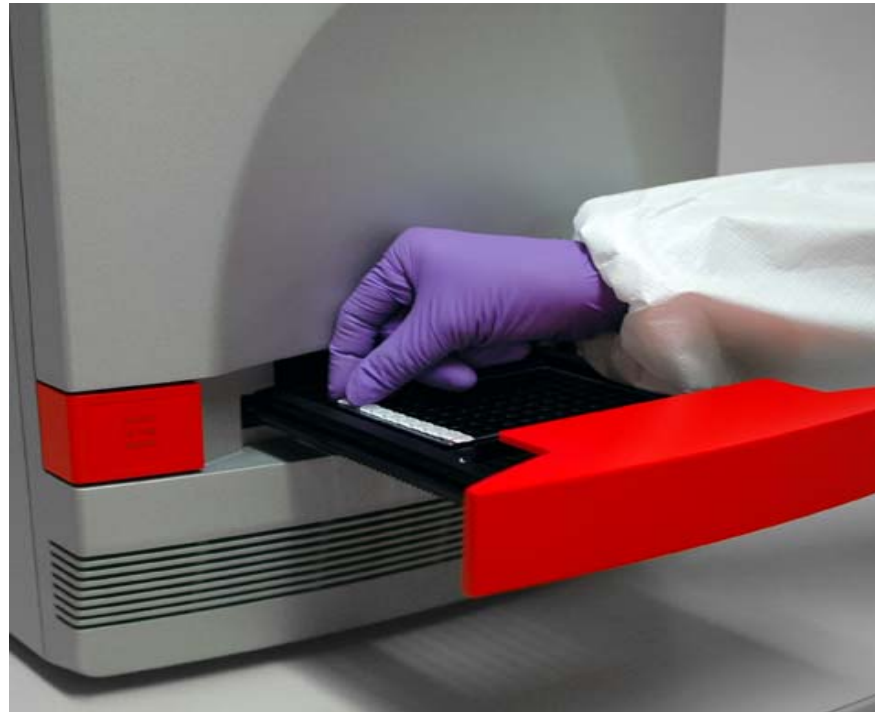
# Sample preparation: *Alphitobias diaperinus* panzer



# Transfer (5 $\mu$ l) in 200 $\mu$ l lysis buffer



# Starting the PCR





# Results I

Sample	PCR		Culture
	Enrichment	Direct-PCR	
Caecal content chicken	neg	neg	neg
Caecal content chicken	neg	neg	neg
Caecal content chicken	neg	neg	neg
Caecal content chicken	neg	neg	neg
Alphitobias diaperinus	neg	neg	n.a.
beetle larvae	neg	neg	n.a.
water	neg	neg	n.a.



# Results II

Sample	Direct-PCR		culture
	species	CFU/ml	
cloaca swab 1	<i>C. jejuni</i>	5,9 x 10 <sup>6</sup>	pos
cloaca swab 2	<i>C. jejuni</i>	< 10 <sup>4</sup>	pos
cloaca swab 3	<i>C. jejuni</i>	2,4 x 10 <sup>6</sup>	pos
cloaca swab 4	<i>C. jejuni</i>	3,1 x 10 <sup>6</sup>	pos
cloaca swab 5	<i>C. jejuni</i>	1,9 x 10 <sup>7</sup>	pos
<p>All samples have been analysed without pre-enrichment (Direct-PCR)</p> <p>No PCR Inhibition</p>			
caecal contents 2a	<i>C. jejuni</i>	4,7 x 10 <sup>5</sup>	pos
caecal contents 2b	<i>C. jejuni</i>	1,6 x 10 <sup>5</sup>	pos
caecal contents 3a	<i>C. jejuni</i>	4,2 x 10 <sup>6</sup>	pos
caecal contents 3b	<i>C. jejuni</i>	2,7 x 10 <sup>5</sup>	pos
caecal contents 4a	<i>C. jejuni</i>	1,5 x 10 <sup>6</sup>	pos
caecal contents 4b	<i>C. jejuni</i>	1,1 x 10 <sup>5</sup>	pos
broiler carcass	<i>C. jejuni</i>		pos



# Results III

## Breeding period up to 4 days after thinning

		days of breeding						
		1-5 / 14 / 19 / 26	29/30 thinning			34		
		PCR enrich.	Direct-PCR	PCR enrich.	culture	Direct-PCR	PCR enrich.	culture
<b>Broiler flock</b>	51	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	52	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	53	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	54	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	55	neg.						
	56	neg.						
	57	neg.						
	58	neg.						



# Results IV

## Sample collection 5 and 6 days after thinning

	D-PCR	PCR-Enr.	culture	D-PCR	PCR-Enr.	culture	D-PCR	PCR-Enr.	culture
flock	first sector			central sector			last sector		
51	neg.	pos.	neg.	neg.	neg.	neg.	neg.	pos.	neg.
52	neg.	pos.	neg.	neg.	pos.	neg.	neg.	pos.	neg.
53	neg.	neg.	neg.	neg.	pos.	neg.	neg.	neg.	neg.
54	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
55	neg.	neg.	neg.	neg.	pos.	neg.	neg.	neg.	neg.
56	neg.	neg.	neg.	neg.	pos.	neg.	neg.	pos.	neg.
57									
58	neg.	pos.	neg.	neg.	pos.	neg.	neg.	neg.	neg.



## Summary

- Easy handling (Few manual steps)
- Fast and reliable (international validated method)
- No limitation with regard to food matrix (spices and animal feed)
- Multiplex-PCR for the detection and identification of Campylobacter
- Direct PCR for the detection of Campylobacter from caecal content is possible



Thank you for your attention

