

## Supplementary material 3

**Appendix table 1. Sample size, enrichment and selective medium used for detection of *Campylobacter* spp.**

Lab code	Sector	Sample size	Enrichment				Selective medium					
			Yes/No	Medium	Temperature	Period	Medium1	Medium2	Temperature	Period	Atmosphere	
L1	F + V	10 mL**	No				mCCDA			41.5°C	48 h	Microaerophilic
L2	F + V	10 mL	Yes	Bolton	41.5°C	48 h	mCCDA	Karmali		41.5°C	48 h	Microaerophilic
L3	F + P	10 mL	Yes	Bolton			mCCDA	Non-selective blood agar		42°C, 37°C	48 h	Microaerophilic
L4	P	20 µL	No				mCCDA			37°C	48 h	Microaerophilic
L5	V	10 µL	Yes	Preston	41.5°C	48 h	mCCDA	CAT		41.5°C	48 h	Microaerophilic
L6	F	10 mL	Yes	Bolton	41.5°C	48 h	mCCDA			41.5°	48 h	Microaerophilic
L7	P	100 µL	No				CUP			37°C	72 h	Microaerophilic
L8	P	10 µL	No				mCCDA			42°C	48 h	Microaerophilic
L9	F + V	NA*	NA*	NA*	NA*	NA*	NA*	NA*		NA*	NA*	NA*
L10	F	10 mL	Yes	Preston	41.5°C	24 h	mCCDA	Butzler		41.5°C	48 h	Microaerophilic
L11	V	10 mL	Yes	Preston	41.5°C	24 h	mCCDA	Butzler		41.5°C	48 h	Microaerophilic
L12	P	10 µL	Yes				Nonselective plate with cefuroxime***			42°C	48 h	Microaerophilic
L13	P	10 µL	No									
L14	F	10 µL	Yes	Preston	41°C	24 h	mCCDA			41°C	48 h	Microaerophilic
L15	F	NA*	NA*	NA*	NA*	NA*	NA*	NA*		NA*	NA*	NA*

V=animal health sector, F=food safety, P=public health; \*Analysis not performed, \*\*A wet swab from the samples was streaked onto selective media, \*\*\* Culture of positive PCR samples only

CAT: Cefoperazone amphotericin teicoplanin; mCCDA: Modified charcoal cephaloperazone deoxycholate agar

**Appendix table 2. Species identification of *Campylobacter* spp.**

Lab code	Sector	Species identification	If PCR, reference	Comments
L1	F + V	PCR	Multiplex PCR (Ugarte-Ruiz et al. 2012)	If suspected colonies were found, streaked them onto blood agar 37°C for 48 h following microaerobic conditions
L2	F + V	MALDI-TOF		
L3	F + P	PCR	Multiplex PCR (Denis et al. 1999)	
L4	P	MALDI-TOF		
L5	V	MALDI-TOF		
L6	F	Biochemical tests		From mCCDA to Blood Agar for confirmation.
L7	P	MALDI-TOF, WGS		
L8	P	Biochemical tests, MALDI-TOF, Microscopy		
L9	F + V	NA*	NA*	
L10	F	Biochemical tests, PCR	Multiplex PCR (Wang et al. 2002)	ISO 10272-1:2017
L11	V	MALDI-TOF	ISO 10272-1	
L12	P	MALDI-TOF, PCR	In-house PCR, target genes <i>ceuE</i> ( <i>C. coli</i> ) and <i>mapA</i> ( <i>C. jejuni</i> )	
L13	P	MALDI-TOF, PCR	BD MAX system	
L14	F	WGS		
L15	F	NA*	NA*	

V=animal health sector, F=food safety, P=public health; \*Analysis not performed

mCCDA: Modified charcoal cephaloperazone deoxycholate agar

Ugarte-Ruiz M, Gómez-Barrero S, Porrero MC, Alvarez J, García M, Comerón MC, Wassenaar TM, Domínguez L. Evaluation of four protocols for the detection and isolation of thermophilic *Campylobacter* from different matrices. J Appl Microbiol. 2012 Jul;113(1):200-8. doi: 10.1111/j.1365-2672.2012.05323.x.

Denis M, Soumet C, Rivoal K, Ermel G, Blivet D, Salvat G, Colin P. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett Appl Microbiol. 1999 Dec;29(6):406-10. doi: 10.1046/j.1472-765x.1999.00658.x.

Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL, Rodgers FG. 2002. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. J Clin Microbiol. 40(12):4744-4747. doi: 10.1128/JCM.40.12.4744-4747.2002.

ISO 10272-1:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 1: Detection method.

**Appendix table 3. Sample size, enrichment and selective medium used for detection of *Salmonella* spp.**

Lab code	Sector	Sample size	Pre-enrichment				Enrichment				Selective medium			
			Yes/No	Medium	Temperature	Period	Yes/No	Medium	Temperature	Period	Medium1	Medium2	Temperature	Period
L1	F + V	10 mL	Yes	BPW	37°C	16-20 h	Yes	MSRV	41.5°C	24 h±3 up to 48h±3	XLD	SMID2	37°C	24 h±3
L2	F + V	10 mL	Yes	BPW	37°C	24 h	Yes	MSRV	41.5°C	24 h	XLD		37°C	24 h
L3	F + P	25 mL	Yes	BPW			Yes	RV, MKTTn	42°C (RV)/ 37°C (MKTTn)	24 h	XLD	Chrom- agar	37°C	
L4	P	20 µL	No				Yes	Selenite	37°C	24 h	Red agar		37°C	24 h
L5	V	25 mL	Yes	BPW	37°C	24 h	Yes	MSRV	41.5°C	24 h	XLD	BGA	37°C	24 h
L6	F	10 mL	Yes	BPW	37°C	24 h	Yes	RV	42°C	24 h	XLD	BGA		
L7	P	100 µL	No				No				Basic agar	DC agar	37°C	24 h
L8	P	10 µL	No				Yes	RV	42°C	24 h	XLD		35°C	24 h
L9	F + V	10 mL	Yes	BPW	37°C	16-20 h	Yes	MSRV	41.5°C	24 h±3	XLD, BGA, novo	Rambach	37°C	24 h±3
L10	F	25 mL	Yes	BPW	37°C	24 h	Yes	MSRV, MKTTn	41.5°C	24 h	XLD	XLT-4	37°C	24 h
L11	V	10 mL	Yes	BPW	37°C	24 h	Yes	MSRV	41.5°C	24 h	XLD	BGA	37°C	24h
L12	P	10 µL	No				Yes	RV	40°C		XLD			
L13	P	10 µL	No				No							
L14	F	10 µL	No				Yes	MSRV	41°C	24 h	XLD	BGA	37°C	24 h
L15	F	10 mL	Yes	BPW	37°C	24 h	Yes	MSRV, MKTTn	41.5°C	24 h	XLD	BGA	37°C	24 h

V=animal health sector, F=food safety, P=public health

BGA: Brilliant green agar; BPW: Buffered peptone water; MKTTn: Muller-Kauffmann tetrathionate/novobiocin broth; MSRV: Modified semi-solid Rappaport-Vassiliadis enrichment media; RV: Rappaport-Vassiliadis; XLD: Xylose lysine deoxycholate agar; XLT-4: Xylose lysine tergitol-4

**Appendix table 4. Species identification and determination of serovar of *Salmonella* spp.**

Lab code	Sector	Species identification	Serotyping	Comments
L1	F + V	Biochemical tests	Classical slide agglutination	ISO 6579 (primary production protocol). Confirmation according to ISO 6579-1:2017
L2	F + V	ISO 6579-1:2017-04	ISO 6579-1:2017-04	EN ISO 6579-1:2017-04
L3	F + P		Classical slide agglutination	0,1 ml of pre-enrichment + 10 ml of RVS and 1 ml of pre-enrichment + 10 ml of MKTTn (selective enrichment)
L4	P	Biochemical tests, MALDI-TOF	WGS, classical slide agglutination	
L5	V	Biochemical tests, MALDI-TOF	Classical slide agglutination	
L6	F	Biochemical tests	Classical slide agglutination	Pre-enrichment with BPW for 18-20 h in 37°C. 100 ul transfer to RVS-broth and Incubation for 24 h in a in 42°C water bath. From RVS a loopful is streaked on agar BriS and XLD.
L7	P	MALDI-TOF, WGS	WGS, classical slide agglutination	
L8	P	MALDI-TOF	Classical slide agglutination	Day one: XLD agar (24 h in 35°C) and Rappaport enrichment broth (24 h in 42°C). Day two: cultivation to XLD from Rappaport broth (24 h in 35°C)
L9	F + V		Classical slide agglutination	EN-ISO 6579-1:2017
L10	F	Biochemical tests	Classical slide agglutination	NF U 47-100
L11	V	MALDI-TOF		
L12	P	MALDI-TOF, PCR		In-house PCR
L13	P	PCR		BD MAX system
L14	F	WGS		
L15	F	Biochemical tests, WGS	WGS, classical slide agglutination	EN-ISO 6579-1:2017

V=animal health sector, F=food safety, P=public health

BPW: Buffered peptone water; BriS: Brilliance *Salmonella*-agar; MKTTn: Muller-Kauffmann tetrathionate/novobiocin broth; RVS: Rappaport-Vassiliadis Soy peptone broth; XLD: Xylose lysine deoxycholate agar

ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.

AFNOR NF U47-100. Standard Methods of analysis in animal health - Search by isolation and identification of any specified serovar or serovar(s) of *Salmonella* in the environment of animal production, Paris.

**Appendix table 5. Sample size, enrichment and selective medium used for detection of *Yersinia enterocolitica*.**

Lab code	Sector	Sample size	Pre-enrichment				Enrichment				Selective medium					
			Yes/No	Medium	Temperature	Period	Yes/No	Medium	Temperature	Period	Medium 1	Temperature	Period	Medium 2	Temperature	Period
L1	F + V	10 mL	Yes	PSB	25°C	5 days	Yes	ITC	25°C	48 h	YER	30°C	48 h	SSDC	30°C	48 h
L2	F + V	1 mL	Yes	PSB	5°C	14 d	Yes				CIN	29°C	24 h			
L3	F + P	25 mL	No				Yes	PSB	25°C	48 h	CIN	30°C	48h	Chromogenic Yersinia agar	30°C	48 h
L4	P	20 µL	No				No				CIN			SSI Diagnostica Red agar plate	37°C	24 h
L5	V	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
L6	F	10 mL	Yes				Yes	PSB	25°C	3 h	CIN	30°C	24 h			
L7	P	100 µL	No				No				Basic agar	37°C	24 h	DC agar	37°C	24 h
L8	P	10 µL	No				No				CIN	30°C (day1) RT (day2)	24 h + 24 h			
L9	F + V	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
L10	F	25 mL	No				Yes	PSB/ITC	25°C	48 h	CIN	30°C	24 h			
L11	V	10 mL	No				Yes	ITC	22°C	48 h	CIN	30°C	48 h			
L12	P	10 µL	No				No				CIN	30°C	24-48 h			
L13	P	10 µL	No				No									
L14	F	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
L15	F	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*

V=animal health sector, F=food safety, P=public health; \*Analysis not performed

CIN: Cefsulodin irgasan novobiocin agar; ITC: Irgasan ticarcillin potassium chlorate broth; PSB: Peptone sorbitol bile salts broth; SSDC: *Salmonella-Shigella*-desoxycholate-calcium chloride

**Appendix table 6. Species identification and determination of biotype of *Yersinia enterocolitica*.**

Lab code	Sector	Species identification	Comments
L1	F + V	Biochemical tests, MALDI-TOF	Direct plating: 1 ml of the sample was streaked onto three plates of agar YER. These plates were incubated at 30°C for 48 h. Suspected colonies were streaked onto blood agar being incubated at 30°C for 48 h. Enrichment on PSB (peptone, sorbitol and bile salts): 10 ml of the sample were diluted onto 90 ml of PSB and incubated at 25°C during 5 days. After this incubation, 25 µl was streaked onto agar YER being incubated at 30°C for 48 h. Suspected colonies were streaked onto blood agar being incubated at 30°C for 48 h. Enrichment on ITC: 10 ml of the sample diluted on PSB (before being incubated) were diluted onto 90 ml of ITC (1/100 from the original sample) and incubated at 25°C during 48h. After this incubation, 25 µl were streaked onto agar SSDC being incubated at 30°C for 48 h. Suspected colonies were streaked onto blood agar being incubated at 30°C for 48 h.
L2	F + V	MALDI-TOF	Pre-enrichment: 10 ml of sample added into 90ml PSB broth, incubated at 5°C (14 days), 10 µl subculture suspended in solution 0.25% KOH in 0.5% NaCl, mixed and one loopful (1 µL loop) was streaked over the surface of CIN agar (29°C, 24h)
L3	F + P	Biochemical tests, PCR	ISO 18867 (Real time PCR for <i>Y. enterocolitica</i> - target ail gene); real time PCR for <i>Y. enterocolitica</i> - <i>ystA</i> and <i>ystB</i> genes
L4	P	MALDI-TOF, Bio/serotyping	
L5	V	NA*	
L6	F	Biochemical tests, Bio-/serotyping	PSB (for 3 h) at 25°C. PSB_> 10 µL and 100 µL onto CIN agar. Incubation at 30°C for 24h. PSB is incubated at 4°C. Day 2: from CIN to BS. Day 8: 100 µL PSB to 10 mL MRB incubated at 25°C for 4 days. Day 12: MRB to CIN and day 13 to BS.
L7	P	MALDI-TOF, WGS	
L8	P	MALDI-TOF, esculin	
L9	F + V	NA*	
L10	F	Biochemical tests	First, streaking of typical colonies (from CIN) on YeCM to differentiate non-pathogenic Ye (BT1A) from pathogenic YE, then biotyping with ISO 10273:2017
L11	V	MALDI-TOF, esculin	
L12	P	MALDI-TOF, esculin, PCR	In-house PCR target ail gene for <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i> .
L13	P	PCR	BD MAX system
L14	F	NA*	

L15 F NA\*

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V=animal health sector, F=food safety, P=public health; \*Analysis not performed

BS: Bromthymol blue saccharose; CIN: Cefsulodin irgasan novobiocin agar; ITC: Irgasan ticarcillin potassium chlorate broth; MRB: Modified Rappaport broth; PSB: Peptone sorbitol bile salts broth; SSDC: *Salmonella-Shigella*-desoxycholate-calcium chloride; YeCM: *Y. enterocolitica* chromogenic medium

ISO/TS 18867:2015 Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

ISO 10273:2017 Microbiology of the food chain — Horizontal method for the detection of pathogenic *Yersinia enterocolitica*