Summary Report for MicroVal certificate 2011LR39, concerning the **food**proof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27) for detection of *Salmonella* in Infant formula and infant cereals, Probiotics containing products, Ingredients and Environmental samples

MicroVal study number: 2011LR39

Alternative methods: the **food**proof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27)

Summary Report: final version 28-01-2019

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This report is prepared in accordance with ISO 16140-2:2016 and MicroVal technical committee interpretation of ISO 16140-2 v.1.0

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Kit names:
foodproof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27) in combination with the **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07) for manual DNA extraction, or the **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15) for automated DNA extraction

Validation standard:
EN ISO 16140-2:2016 Microbiology of the food chain – Method validation – Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

Reference methods:
EN 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.

EN ISO 6579:2002; Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

Scope of validation:
Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

Certification organization: Lloyd's Register

List of abbreviations / legends

A(lt)	Alternative method
AA	Alpha-amylase added to initial BPW (at 50 mg per 100 gram sample)
AL	Acceptability Limit
Art. Cont.	artificial contamination
BPW	Buffered Peptone Water
BV	Vancomycin added to initial BPW (at 10 mg/l)
CFU	Colony Forming Units
D	Double-concentrated BPW
DV	Double-concentrated BPW plus vancomycin (at 10 mg/l)
EB	*Enterobacteriaceae*
EB/C	*Enterobacteriaceae* / *Cronobacter*
EBC test kit	PCR test kit for detection of *Enterobactereriaceae* and *Cronobacter*
EL	Expert Laboratory
FP	False Positive
FPR	False Positive Ratio
g	Gram
h	Hour
ILS	Interlaboratory Study
Inh	inhibition
LDC	l-Lysine decarboxylation medium
LOD	Level of Detection
MCS	Method Comparison Study
min	minute
MKTTn	Muller-Kauffmann tetrathionate-novobiocin broth
ml	millilitre
MSRV	Modified Semi-solid Rappaport Vassiliadis medium
MVTC	MicroVal Technical Committee
NA	Negative Agreement
na	not applicable
NuA	Nutrient Agar
ND	Negative Deviation
neg (-)	negative/no growth/no reaction/target not detected
ng	no growth at all
ns	Non-Suspect growth
nt	not tested
PA	Positive Agreement
PD	Positive Deviation
pos (+)	positive/growth/target detected
PPNA	Presumptive Positive Negative Agreement (belongs to the False Positive results)
PPND	Presumptive Positive Negative Deviation (belongs to the False Positive results)
R(ef)	Reference method
rep	repetition
rep(*neg)	repetition indicated by the Diagnostic Interpreter system, but negative by personal interpretation of the curve
rep(*pos)	repetition indicated by the Diagnostic Interpreter system, but positive by personal interpretation of the curve
RIVM	National Institute for Public Health and the Environment
RLOD	Relative Level of Detection
r/r	repetition/repetition (first result: repetition/second result: repetition)
RVS	Rappaport-Vassiliadis Soya broth
RT	Relative Trueness
S	Suspect growth
S (n.c.)	Suspect growth, but negative confirmed after the confirmation steps

SE	Relative Sensitivity
SP	Relative Specificity
S test kit	PCR test kit for detection of *Salmonella*
TP	True Positive
TSA	Tryptone Soya Agar
TSI	Triple Sugar Iron agar
UA	Urea Agar
XLD	Xylose Lysine Deoxycholate agar
Z&O	Centre for Zoonoses and Environmental Microbiology

For easier recognition, the following colour-codes were used throughout the report:

	Reference method	Reference method
	StarPrep One Kit	StarPrep One Kit
	MagPrep IV	Magnetic Preparation Kit IV
	PCR kit A	PCR kit A
	PCR Kit B	PCR Kit B
	kit A/B	PCR Kit A and PCR kit B
P1	3 h 2nd BPW	3 h incubation of second BPW
P2	20 h 2nd BPW	20 h incubation of second BPW
	Totals	Totals
	ND, PD	ND, PD, deviating results

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1. Introduction

In this project an **extension** of MicroVal certificate 20011LR39 (detection of *Salmonella* spp., after screening for presence of *Enterobacteriaceae*, RIVM Confidential Reports 330634001/2012 and 330634002/2012) was carried out by RIVM-Z&O as the MicroVal Expert laboratory.
The extension to this certificate basically concerned the ***DNA extraction step*** (**food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15), in combination with the KingFisher® Flex instrument, as an automated extraction step).

In addition, this extension also included:
-New Category: Environmental samples, using the original (manual) DNA extraction method (**food**proof® StarPrep One, BIOTECON Diagnostics Cat. No. S 400 07).
-New test protocol for the Category Probiotics containing products: 3-4 h incubation of the second enrichment step in BWP using the original (manual) DNA extraction step (**food**proof® StarPrep One, BIOTECON Diagnostics Cat. No. S 400 07).

To align with all 4 Categories as concurrently tested for the closely related study LR 8/9/19/20 (detection of *Enterobacteriaceae* and/or *Cronobacter* spp.), all (joint) samples within the sensitivity study and the RLOD study were tested also within the LR 39 study.

Meanwhile, also the **renewal** of MicroVal certificate 2011LR39 was carried out by RIVM-Z&O as the MicroVal Expert Laboratory. The original validation study for certificate LR 39 concerning 3 categories (2012, RIVM Confidential Reports 330634001/2012 and 330634001/2012) was elaborated according to ISO 16140:2003. All available data were re-evaluated according to ISO 16140-2:2016, and the validation data were confirmed to be valid.

This report combines the applicable data from both the extension study and the renewal study into one complete overview on the current situation for MicroVal certificate LR 39.

The alternative methods are:

Alternative method **PCR test kits A**:
* the **food**proof® *Salmonella* Detection Kit, - **Hybridization probes** - (BIOTECON Diagnostics Cat. No. R 310 27); after pre-screening by using * the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit, **-Hybridization probes-** (BIOTECON Diagnostics Cat. No. R 310 15.1);

in combination with the following necessary kits and reagents for DNA extraction:

- Manual DNA extraction:
  - Reagent D (BIOTECON Diagnostics Cat. No. A 500 02)
  - the **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07)

Or

- Automated DNA extraction:
  - Reagent D (BIOTECON Diagnostics Cat. No. A 500 02)
  - the **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15)

Alternative method **PCR test kits B**:
* the **food**proof® *Salmonella* Detection Kit, - **5'Nuclease** - (BIOTECON Diagnostics Cat. No. R 302 27); after pre-screening by using * the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit, **-5' Nuclease-** (BIOTECON Diagnostics Cat. No. R 302 15.1);

in combination with the following necessary kits and reagents for DNA extraction:

- Manual DNA extraction:
  - Reagent D (BIOTECON Diagnostics Cat. No. A 500 02)
  - the **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07)

Or

- Automated DNA extraction:
  - Reagent D (BIOTECON Diagnostics Cat. No. A 500 02)
  - the **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15)

The reference method is:
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.

The validation studies were carried out using the reference method as described in ISO 6579:2002; Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.. A new version of ISO 6579 was published in 2017, and the main changes introduced in the 2017 version compared to ISO 6579:2002 are considered as minor. Therefore, the validation data can be considered valid for both version ISO 6579:2002 and version ISO 6579-1:2017.

Scope of the validation study (Categories):

Original Certificate 2011LR39	2018 Certificate LR39
Powdered infant formula	Infant formula and infant cereals
Probiotic culture powders/pre-blends	Probiotics containing products
Ingredients	Ingredients
	Environmental samples

Criteria evaluated during the Extension/Renewal studies have been:

- Method Comparison Study (MCS)
  - Sensitivity study
  - Relative level of detection study
  - Inclusivity and exclusivity study
- Interlaboratory Study (ILS)

Overall, the conclusions for the Method Comparison Study and the Interlaboratory Study are:

The alternative method foodproof® *Salmonella* Detection Kit (Hybridization probes and 5'Nuclease), using the foodproof® StarPrep One Kit for manual DNA extraction, shows comparable performance to the reference method ISO 6579-1:2017 for the detection of *Salmonella* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Salmonella* Detection Kit (Hybridization probes and 5'Nuclease), using the foodproof® Magnetic Preparation Kit IV for automated DNA extraction, shows comparable performance to the reference method ISO 6579-1:2017 for the detection of *Salmonella* in Infant formula and infant cereals, and in Probiotics containing products.

The scope for the **food**proof® Magnetic Preparation Kit IV for automated DNA extraction is **restricted** to Infant formula and infant cereals, and Probiotics containing products, the latter needing the specific sub-cultivation protocol (second BPW for 20 h at 37°C).

The overview on the claims for the current LR39 is given in the Table below:

Detection of *Salmonella*

	Enrichment	Standard protocol: 100 g sample material* plus 900 ml BPW pre-warmed at 37 °C, 16 - 20 h at 37 °C ± 1 °C									
	Subcultivation	Standard protocol P1: 100 µl of BPW culture in 900 µl BPW, 3 - 4 h at 37 °C ±1 °C (shaking at 900 U/min)									
		Specific protocol P2: 100 µl of BPW culture in 900 µl BPW, 20 - 24 h at 37 °C ± 1 °C (shaking at 900 U/min)									
	Reagent D treatment	A 500 02									
	DNA extraction	Manual protocol **food**proof StarPrep One Kit					Automated protocol **food**proof MagPrep IV Kit				
		S 400 07					S 400 15				
	Real-time PCR, pre-screening on EB	**food**proof EBC detection kit, Hybridization probes		**food**proof EBC detection kit, 5'Nuclease			**food**proof EBC detection kit, Hybridization probes		**food**proof EBC detection kit, 5'Nuclease		
		R 310 15.1		R 302 15.1			R 310 15.1		R 302 15.1		
	Real-time PCR, *Salmonella* detection	**food**proof S detection kit, Hybridization probes		**food**proof S detection kit, 5'Nuclease			**food**proof S detection kit, Hybridization probes		**food**proof S detection kit, 5'Nuclease		
		R 310 27		R 302 27			R 310 27		R 302 27		
	Thermocyclers	LC 480 II	LC 2.0	LC 480 II	IQ5	Mx 3005	LC 480 II	LC 2.0	LC 480 II	IQ5	Mx 3005
	Software version	1.5.1	4.1	1.5.1	2.0	MX4.1d	1.5.1	4.1	1.5.1	2.0	MX4.1d
Scope (including subcultivation Protocol)	Infant formula and infant cereals (P1)	X	X	X	X	X	X	X	X	X	X
	Probiotics containing products (P1)	X	X	X	X	X					
	Probiotics containing products (P2)	X	X	X	X	X	X	X	X	X	X
	Ingredients (P1)	X	X	X	X	X					
	Environmental samples (P1)	X	X	X	X	X					

*specific sample preparations: see Annex C or EBC detection kit inserts

2. Method Protocols

2.1 Reference method

See the flow diagram of the reference method in Annex A.

2.2 Alternative methods

See the flow diagram of the alternative methods in Annex B.

In short, the alternative method is using "reagent D" treatment (BIOTECON Diagnostics Cat. No. A 500 02) for rapid elimination of DNA from dead bacterial cells to avoid false-positive PCR results.
The **food**proof® StarPrep One Kit is used for manual extraction of DNA from Gram-negative bacteria like *Enterobacteriaceae* for direct use in PCR.
The **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15), in combination with the KingFisher® Flex instrument, is used for automated DNA extraction.
Presence of *Enterobacteriaceae* is tested by real-time PCR: test kit BIOTECON Diagnostics Cat. No. R310 15.1 (Hybridization probes) and test kit BIOTECON Diagnostics Cat. No. R 302 15.1 5'Nuclease).
Samples positive for *Enterobacteriaceae* are next tested on presence of *Salmonella* by *Salmonella*-specific real-time PCR: test kit BIOTECON Diagnostics Cat. No. R 310 27 (Hybridization probes) and test kit BIOTECON Diagnostics Cat. No. R 302 27 (5'Nuclease).
The **micro**proof Diagnostic Interpreter software is used for easy handling and interpretation of the PCR results.

The various kit inserts are given in the following Annexes:

Annex D: Kit insert reagent D (BIOTECON Diagnostics Cat. No. A 500 02; version 3, April 2018).

Annex E: Kit insert **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07; version 6, November 2018).

Annex F: Kit insert **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15; version 1, August 2014).

Annex G: Kit insert EBES test kit A: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - Hybridization probes - (BIOTECON Diagnostics Cat. No. R 310 15.1; version 5, October 2018, as updated according to MicroVal rules).

Annex H: Kit insert EBES test kit B: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - 5'Nuclease - (BIOTECON Diagnostics Cat. No. R 302 15.1; version 3, November 2018, as updated according to MicroVal rules).

Annex I: Kit insert S test kit A: **food**proof® *Salmonella* Detection Kit - Hybridization probes - (BIOTECON Diagnostics Cat. No. R 310 27;version 5, October 2018, as updated according to MicroVal rules).

Annex J: Kit insert S test kit B: **food**proof® *Salmonella* Detection Kit – 5'Nuclease - (BIOTECON Diagnostics Cat. No. R 302 27; version 5, November 2018, as updated according to MicroVal rules).

NB: Study LR 39 and Study LR 8/9/19/20 are closely related and have the Annexes D – H on the kit inserts in common.

2.3 Study design

Both the original validation study 2011LR39 and the extension study were carried out using 100 gram test portions of sample material, because that is the sample size widely used in routine testing laboratories. The validation data are considered also to be valid for smaller amounts of samples (e.g. 10 or 25 gram), as long as the (minimum) primary dilution 1 in 10 of the sample in BPW is used.
A question on the reference method was put forward in the protocol-approval phase of the study (2016), concerning the use of vancomycin when testing probiotics containing samples as indicated in the protocol of the alternative method under study. No sample preparation for probiotic containing products was given in the reference method or relevant part of ISO 6887 at the time. However, the ISO reference method is stating: “prepare the test samples in accordance with the relevant part of ISO 6887 dealing with the product concerned. If ISO 6887 is not appropriate, it is recommended that the parties concerned come to an agreement on this subject.” Therefore it could be decided to use vancomycin for the reference method as well (for the probiotic containing samples). Moreover, vancomycin does not impair growth, it was used in the original validation study and adding would be more challenging for the alternative method. After discussing this question, the MVTC decided (30th meeting) that the study can be conducted as a **paired** study. Nowadays, ISO 6887-4:2017 is allowing the addition of antibiotics when testing samples containing probiotics, and section 9.8.2 referring to ISO 27205 which specifically describes the use of vancomycin.
The reference and the alternative methods were performed with the same test portion, starting with the same specific pre-enrichment in BPW (see Annex C). This guidance on specific sample preparations was also taken up in the relevant test kit inserts of the alternative methods.
Method descriptions and kit inserts of the various steps in the alternative methods are given in the Annexes D-J. The **micro**proof Diagnostic Interpreter software was used for easy handling and interpretation of the PCR results. In case of a “repetition” result, guidelines as now given in the updated kit inserts were followed.

For the extension study, the Expert Laboratory performed the PCR tests (kit A, hybridization probes) on a LightCycler 480 II real-time PCR system (Roche), using the **micro**proof Diagnostic Interpreter software for analysis of the PCR data and data interpretation. Additionally, test kit B (5’Nuclease) was validated by an external laboratory (BIOTECON Diagnostics, Potsdam, Germany) using a LightCycler 480 II system, on blindly-coded DNA samples.
In the original study, the Expert laboratory performed the PCR tests (kit A, hybridization probes) on a LightCycler® 2.0 real-time PCR system (Roche). Additionally, the complete method comparison study was also validated using the LC 480 II (Roche) PCR machine for test kit A and using the PCR machines LC 480 II (Roche), Mx3005p (Agilent) and iQ5 (Bio-Rad) for test kit B, based on testing blindly-coded DNA samples by an external laboratory (BIOTECON Diagnostics, Potsdam, Germany). The raw data on the various additional PCR machines from the original study report are given in Annex Q for information.

Note that the practical work of the extension study on 2011LR39 was combined with the extension study on MicroVal certificate 2009LR8/9/19/20 (BIOTECON alternative methods for detection of *Enterobacteriaceae (EB)* and/or *Cronobacter* (C)), because the same set of samples were used for both detection of EB and/or *Cronobacter* as well as detection of *Salmonella* after the pre-screening for EB(/C).

3. Method comparison study

3.1 Sensitivity study

The sensitivity study (SE) is the ability of the method selected to detect the analyte by either the reference or the alternative method.

3.1.1 Categories and sample types

A total of 4 Categories were included in this validation study.
A minimum of 60 Items for each Category were tested by both the reference methods and the alternative methods in the sensitivity study, with a minimum of 30 positive samples per Category.
Each Category was made up of 3 Types, with at least 20 Items representative for that Type.

The categories, the types and the number of samples analyzed are presented in Table 1 applicable to both the StarPrep One Kit and the MagPrep IV.

Table 1 - Categories, types and number of samples analysed, applicable to both the StarPrep One Kit and the MagPrep IV (PCR test kit A and test kit B).

Category		Type		Alternative method protocols[1]		Test portion size	Number of samples tested
				1	2		
1	Infant formula and infant cereals	a	Infant formula (intended for infants < 1 year)	x		100 g	22
		b	Infant formula (intended for infants > 1 year)	x		100 g	21
		c	Infant cereals	x		100 g	21
2	Probiotic containing products	a	Probiotic infant formula	x	x	100 g	23
		b	Probiotic infant cereals	x	x	100 g	22
		c	Probiotic ingredients	x	x	100 g	20
3	Ingredients	a	Standard ingredients	x		100 g	22
		b	Infant cereals ingredients	x		100 g	22
		c	Premixes	x		12,5 g	25
4	Environmental samples	a	Sweep samples/equipment swabs	x		swabs	24
		b	Traject samples (in-line factory)	x		100 g	22
		c	Vacuum cleaner residues	x		100 g	20

[1] Alternative method protocols:

1	3 h incubation of second BPW
2	20 h incubation of second BPW

A total of 264 samples were analyzed. The distribution of positive and negative samples per tested category and type is given in Table 2, applicable to both the StarPrep One Kit and the MagPrep IV and identical for PCR test kits A and PCR test kits B, except for 1 missing value as indicated.

Table 2 - Distribution per tested category and type: applicable to both the StarPrep One Kit and the MagPrep IV (PCR test kit A and PCR test kit B).

Category		Type	Positive* samples	Negative samples	Total
1	Infant formula and infant cereals	a	11	11	22
		b	9	12	21
		c	10	11	21
		Total	30	34	64
2	Probiotic containing products	a	11	12	23
		b	11	11	22
		c	9	11	20
		Total	31	34	65
3	Ingredients	a	10	12	22
		b	10	12	22
		c	12	13	25
		Total	32	37	69
4	Environmental samples	a	11	13	24
		b	10[1]	12	22
		c	11	9	20
		Total	32	34	66
Total			125	139	264

*Positive by at least one of the methods
[1] One missing value for test kit B, MagPrep Kit IV.

3.1.2 Test sample preparation

None of the samples tested were naturally contaminated with *Salmonella.*

Naturally contaminated samples would preferentially be analyzed, but due to the nature of the samples and the target organism it was clear that artificial contaminations were needed.

The artificial contaminations are presented in Annex K. Note that generally the same samples were used for both the LR39 study and the closely related LR 8/9/19/20 study, therefore using artificial inoculations with combinations of *Salmonella* and *Cronobacter* strains.
Generally, a seeding protocol was used, adding a pinch of skim milk powder-dried strains to the test samples and leaving this at room temperature for a minimum of 2 weeks before actual testing. In some cases a *Salmonella* lenticule (drying/freezing stress) was used for direct inoculation (spiking).

150 samples were artificially contaminated with *Salmonella*, using 38 different strains. 125 gave a positive result for *Enterobacteriaceae*, followed by a positive result for *Salmonella*.

The same strain (or strain combination) was not used to inoculate more than 6 samples.

3.1.3 Confirmation protocols

Confirmation of *Salmonella* was done according to ISO 6579 (serological; biochemical: TSI, UA, LDC).
Samples showing discordant results were re-tested by using the cold-stored initial BPW enrichment, both directly and as another test portion of 10 ml submitted again to ISO 6579 (RVS, MKTTn, additionally MSRV).

3.1.4 Sensitivity study results

All raw data on the sensitivity study are given in Annex L. Sample numbers in bold indicate artificial inoculation of the sample (see Annex K for details on artificial inoculation).

The summary of results of the reference method and the alternative methods, the StarPrep One Kit and the MagPrep IV, for all Categories, and PCR test kit A and test kit B, are given in Tables 3 (Categories 1, 2.1, 3, and 4) and Tables 4 (Categories 1, 2.2, 3, and 4).

Table 3-S/Table 4-S - Summary of sensitivity study results: StarPrep One Kit, all categories (1, 2.1 or 2.2, 3, 4)

Test kit:		Reference method positive (R+)	Reference method negative (R-)
A/B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 121	Positive deviation (R-/A+) PD = 1
A/B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 3	Negative agreement (R-/A-) NA =139

Table 4-M - Summary of sensitivity study results: MagPrep IV all categories (1, 2.1, 3, 4)

Test kit:		Reference method positive (R+)	Reference method negative (R-)
Test kit A	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 114	Positive deviation (R-/A+) PD = 1
Test kit A	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 10	Negative agreement (R-/A-) NA =139
Test kit B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 114	Positive deviation (R-/A+) PD = 1
Test kit B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 9	Negative agreement (R-/A-) NA =139

[1] One missing value for test kit B

Table 4-M - Summary of sensitivity study results: MagPrep IV all categories (1, 2.2, 3, 4)

Test kit:		Reference method positive (R+)	Reference method negative (R-)
Test kit A	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 115	Positive deviation (R-/A+) PD = 1
Test kit A	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 9	Negative agreement (R-/A-) NA =139
Test kit B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 115	Positive deviation (R-/A+) PD = 1
Test kit B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 8	Negative agreement (R-/A-) NA =139

[1] One missing value for test kit B

Tables 5 (PCR test kit A) and Tables 6 (PCR test kit B) show the interpretation of sample results between the reference and alternative method (based on the confirmed alternative method), for each of the alternative method protocols under study.

Table 5-S – Interpretation of sample results: StarPrep One Kit, *PCR test kit A*

Category		Type		PA	NA[1]	PD	ND[2]	PPNA[3]	PPND[3]	Total
1	Infant formula and infant cereals	a	Infant formula (intended for infants < 1 year)	10	11	0	1	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	12	0	0	0	0	21
		c	Infant cereals	9	11	0	1	0	0	21
		Total		28	34	0	2	0	0	64
2.1	Probiotic containing products (Protocol 3 h second BPW)	a	Probiotic infant formula	10	12	0	1	0	0	23
		b	Probiotic infant cereals	11	11	0	0	0	0	22
		c	Probiotic ingredients	9	11	0	0	0	0	20
		Total		30	34	0	1	0	0	65
2.2	Probiotic containing products (Protocol 20 h second BPW)	a	Probiotic infant formula	10	12	0	1	0	0	23
		b	Probiotic infant cereals	11	11	0	0	0	0	22
		c	Probiotic ingredients	9	11	0	0	1	0	20
		Total		30	34	0	1	1	0	65
3	Ingredients	a	Standard ingredients	10	12	0	0	1	0	22
		b	Infant cereals ingredients	10	12	0	0	0	0	22
		c	Premixes	12	13	0	0	0	0	25
		Total		32	37	0	0	1	0	69
4	Environmental samples	a	Sweep samples/equipment swabs	10	13	1	0	0	0	24
		b	Traject samples (in-line factory)	10	12	0	0	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		31	34	1	0	0	0	66
ALL Categories (1, 2.1, 3, 4)				121	139	1	3	1	0	264
ALL Categories (1, 2.2, 3, 4)				121	139	1	3	2	0	264

[1] NA including PPNA, [2] ND including PPND, [3] PPNA + PPND = FP

Table 6-S - Interpretation of sample results: StarPrep One Kit, *PCR test kit B*

Category		Type		PA	NA[1]	PD	ND[2]	PPNA[3]	PPND[3]	Total
1	Infant formula and infant cereals	a	Infant formula (intended for infants < 1 year)	9	11	0	2	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	12	0	0	2	0	21
		c	Infant cereals	9	11	0	1	0	0	21
		Total		27	34	0	3	2	0	64
2.1	Probiotic containing products (Protocol 3 h second BPW)	a	Probiotic infant formula	11	12	0	0	0	0	23
		b	Probiotic infant cereals	11	11	0	0	1	0	22
		c	Probiotic ingredients	9	11	0	0	1	0	20
		Total		31	34	0	0	2	0	65
2.2	Probiotic containing products (Protocol 20 h second BPW)	a	Probiotic infant formula	11	12	0	0	0	0	23
		b	Probiotic infant cereals	11	11	0	0	1	0	22
		c	Probiotic ingredients	9	11	0	0	3	0	20
		Total		31	34	0	0	4	0	65
3	Ingredients	a	Standard ingredients	10	12	0	0	1	0	22
		b	Infant cereals ingredients	10	12	0	0	2	0	22
		c	Premixes	12	13	0	0	0	0	25
		Total		32	37	0	0	3	0	69
4	Environmental samples	a	Sweep samples/equipment swabs	10	13	1	0	2	0	24
		b	Traject samples (in-line factory)	10	12	0	0	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	1	0	20
		Total		31	34	1	0	3	0	66
ALL Categories (1, 2.1, 3, 4)				121	139	1	3	10	0	264
ALL Categories (1, 2.2, 3, 4)				121	139	1	3	12	0	264

[1] NA including PPNA, [2] ND including PPND, [3] PPNA + PPND = FP

Table 5-M – Interpretation of sample results: MagPrep IV *PCR test kit A*

Category		Type		PA	NA[1]	PD	ND[2]	PPNA[3]	PPND[3]	Total
1	Infant formula and infant cereals	a	Infant formula (intended for infants < 1 year)	9	11	0	2	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	12	0	0	0	0	21
		c	Infant cereals	9	11	0	1	0	0	21
		Total		27	34	0	3	0	0	64
2.1	Probiotic containing products (Protocol 3 h second BPW)	a	Probiotic infant formula	9	12	0	2	0	0	23
		b	Probiotic infant cereals	11	11	0	0	0	0	22
		c	Probiotic ingredients	9	11	0	0	0	0	20
		Total		29	34	0	2	0	0	65
2.2	Probiotic containing products (Protocol 20 h second BPW)	a	Probiotic infant formula	10	12	0	1	0	0	23
		b	Probiotic infant cereals	11	11	0	0	0	0	22
		c	Probiotic ingredients	9	11	0	0	0	0	20
		Total		30	34	0	1	0	0	65
3	Ingredients	a	Standard ingredients	8	12	0	2	0	0	22
		b	Infant cereals ingredients	9	12	0	1	0	0	22
		c	Premixes	12	13	0	0	0	0	25
		Total		29	37	0	3	0	0	69
4	Environmental samples	a	Sweep samples/equipment swabs	8	13	1	2	0	0	24
		b	Traject samples (in-line factory)	10	12	0	0	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		29	34	1	2	0	0	66
ALL Categories (1, 2.1, 3, 4)				114	139	1	10	0	0	264
ALL Categories (1, 2.2, 3, 4)				115	139	1	9	0	0	264
Categories 1 and 2.2				57	68	0	4	0	0	129

[1] NA including PPNA, [2] ND including PPND, [3] PPNA + PPND = FP

Table 6-M - Interpretation of sample results: MagPrep IV, *PCR test kit B*

Category		Type		PA	NA[1]	PD	ND[2]	PPNA[3]	PPND[3]	Total
1	Infant formula and infant cereals	a	Infant formula (intended for infants < 1 year)	9	11	0	2	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	12	0	0	0	0	21
		c	Infant cereals	9	11	0	1	0	0	21
		Total		27	34	0	3	0	0	64
2.1	Probiotic containing products (Protocol 3 h second BPW)	a	Probiotic infant formula	9	12	0	2	0	0	23
		b	Probiotic infant cereals	11	11	0	0	1	0	22
		c	Probiotic ingredients	9	11	0	0	0	0	20
		Total		29	34	0	2	1	0	65
2.2	Probiotic containing products (Protocol 20 h second BPW)	a	Probiotic infant formula	10	12	0	1	0	0	23
		b	Probiotic infant cereals	11	11	0	0	0	0	22
		c	Probiotic ingredients	9	11	0	0	1	0	20
		Total		30	34	0	1	1	0	65
3	Ingredients	a	Standard ingredients	8	12	0	2	1	0	22
		b	Infant cereals ingredients	10	12	0	0	0	0	22
		c	Premixes	11	13	0	1	0	0	25
		Total		29	37	0	3	1	0	69
4	Environmental samples	a	Sweep samples/equipment swabs	9	13	1	1	0	0	24
		b	Traject samples (in-line factory)	9	12	0	0	0	0	21
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		29	34	1	1	0	0	65
ALL Categories (1, 2.1, 3, 4)				114	139	1	9	2	0	263
ALL Categories (1, 2.2, 3, 4)				115	139	1	8	2	0	263
Categories 1 and 2.2				57	68	0	4	1	0	129

[1] NA including PPNA, [2] ND including PPND, [3] PPNA + PPND = FP

3.1.5 Sensitivity study calculations

The sensitivity study parameters as specified in Table 7 (ISO 16140-2:2016) were calculated for all Categories and Types, and the overview for each of the alternative method protocols under study is given in Tables 8 (PCR test kit A) and Tables 9 (PCR test kit B). More False Positive results were seen for test kit B than for test kit A (Table 9-S and Table 8-S), using the StarPrepOne Kit, most often due to an initial repetition result. This maybe could have been caused by the fact that the DNA samples to be tested by kit B were transported to an external laboratory before testing.

Table 7– Formula to calculate the sensitivity parameters

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+ND+PD)} \times 100\%$
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+ND+PD)} \times 100\%$
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\%$
False positive ratio for the alternative method	$FPR = \frac{(FP)}{NA} \times 100\%$

Table 8-S – Overview calculated sensitivity parameters per Category and Type:

StarPrep One Kit, *PCR test kit A*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	10	11	0	1	0	91%	100,0%	95,5%	0,0%
	b	9	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	Total	28	34	0	2	0	93,3%	100,0%	96,9%	0,0%
2.1	a	10	12	0	1	0	90,9%	100,0%	95,7%	0,0%
	b	11	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	30	34	0	1	0	96,8%	100,0%	98,5%	0,0%
2.2	a	10	12	0	1	0	90,9%	100,0%	95,7%	0,0%
	b	11	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	0	1	100,0%	100,0%	100,0%	9,1%
	Total	30	34	0	1	1	96,8%	100,0%	98,5%	2,9%
3	a	10	12	0	0	1	100,0%	100,0%	100,0%	8,3%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	12	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	32	37	0	0	1	100,0%	100,0%	100,0%	2,7%
4	a	10	13	1	0	0	100,0%	90,9%	95,8%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	11	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	31	34	1	0	0	100,0%	96,9%	98,5%	0,0%
ALL Cats (with 2.1)		121	139	1	3	1	97,6%	99,2%	98,5%	0,7%
ALL Cats (with 2.2)		121	139	1	3	2	97,6%	99,2%	98,5%	1,4%

[1] NA including PPNA, [2] ND including PPND, [3] FP = PPNA + PPND

Table 9-S - Overview calculated sensitivity parameters per Category and Type: StarPrep One Kit, *PCR test kit B*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	9	11	0	2	0	82%	100,0%	90,9%	0,0%
	b	9	12	0	0	2	100,0%	100,0%	100,0%	16,7%
	c	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	Total	27	34	0	3	2	90,0%	100,0%	95,3%	5,9%
2.1	a	11	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	11	11	0	0	1	100,0%	100,0%	100,0%	9,1%
	c	9	11	0	0	1	100,0%	100,0%	100,0%	9,1%
	Total	31	34	0	0	2	100,0%	100,0%	100,0%	5,9%
2.2	a	11	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	11	11	0	0	1	100,0%	100,0%	100,0%	9,1%
	c	9	11	0	0	3	100,0%	100,0%	100,0%	27,3%
	Total	31	34	0	0	4	100,0%	100,0%	100,0%	11,8%
3	a	10	12	0	0	1	100,0%	100,0%	100,0%	8,3%
	b	10	12	0	0	2	100,0%	100,0%	100,0%	16,7%
	c	12	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	32	37	0	0	3	100,0%	100,0%	100,0%	8,1%
4	a	10	13	1	0	2	100,0%	90,9%	95,8%	15,4%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	11	9	0	0	1	100,0%	100,0%	100,0%	11,1%
	Total	31	34	1	0	3	100,0%	96,9%	98,5%	8,8%
ALL Cats (with 2.1)		121	139	1	3	10	97,6%	99,2%	98,5%	7,2%
ALL Cats (with 2.2)		121	139	1	3	12	97,6%	99,2%	98,5%	8,6%

[1] NA including PPNA, [2] ND including PPND, [3] FP = PPNA + PPND

Table 8-M – Overview calculated sensitivity parameters per Category and Type: MagPrep IV, *PCR test kit A*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	9	11	0	2	0	82%	100,0%	90,9%	0,0%
	b	9	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	Total	27	34	0	3	0	90,0%	100,0%	95,3%	0,0%
2.1	a	9	12	0	2	0	81,8%	100,0%	91,3%	0,0%
	b	11	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	29	34	0	2	0	93,5%	100,0%	96,9%	0,0%
2.2	a	10	12	0	1	0	90,9%	100,0%	95,7%	0,0%
	b	11	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	30	34	0	1	0	96,8%	100,0%	98,5%	0,0%
3	a	8	12	0	2	0	80,0%	100,0%	90,9%	0,0%
	b	9	12	0	1	0	90,0%	100,0%	95,5%	0,0%
	c	12	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	29	37	0	3	0	90,6%	100,0%	95,7%	0,0%
4	a	8	13	1	2	0	81,8%	90,9%	87,5%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	11	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	29	34	1	2	0	93,8%	96,9%	95,5%	0,0%
ALL Cats (with 2.1)		114	139	1	10	0	92,0%	99,2%	95,8%	0,0%
ALL Cats (with 2.2)		115	139	1	9	0	92,8%	99,2%	96,2%	0,0%
Cats 1 and 2b		57	68	0	4	0	93,4%	100,0%	96,9%	0,0%

[1] NA including PPNA, [2] ND including PPND, [3] FP = PPNA + PPND

Table 9-M - Overview calculated sensitivity parameters per Category and Type:

MagPrep IV, ***PCR test kit B***

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	9	11	0	2	0	81,8%	100,0%	90,9%	0,0%
	b	9	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	Total	27	34	0	3	0	90,0%	100,0%	95,3%	0,0%
2.1	a	9	12	0	2	0	81,8%	100,0%	91,3%	0,0%
	b	11	11	0	0	1	100,0%	100,0%	100,0%	9,1%
	c	9	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	29	34	0	2	1	93,5%	100,0%	96,9%	2,9%
2.2	a	10	12	0	1	0	90,9%	100,0%	95,7%	0,0%
	b	11	11	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	9	11	0	0	1	100,0%	100,0%	100,0%	9,1%
	Total	30	34	0	1	1	96,8%	100,0%	98,5%	2,9%
3	a	8	12	0	2	1	80,0%	100,0%	90,9%	8,3%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	11	13	0	1	0	91,7%	100,0%	96,0%	0,0%
	Total	29	37	0	3	1	90,6%	100,0%	95,7%	2,7%
4	a	9	13	1	1	0	90,9%	90,9%	91,7%	0,0%
	b	9	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	11	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	29	34	1	1	0	96,8%	96,8%	96,9%	0,0%
ALL Cats (with 2.1)		114	139	1	9	2	92,7%	99,2%	96,2%	1,4%
ALL Cats (with 2.2)		115	139	1	8	2	93,5%	99,2%	96,6%	1,4%
Cats 1 and 2b		57	68	0	4	1	93,4%	100,0%	96,9%	1,5%

[1] NA including PPNA, [2] ND including PPND, [3] FP = PPNA + PPND

3.1.6 Discordant results

Negative deviations per alternative method protocol are listed in Table 10. Note that some samples showed a negative deviation for more than one alternative method protocol.

Table 10 - Negative deviations

			StarPrep One Kit		MagPrep IV Kit					
Category/ Type	Sample n°	Alt method protocol	Kit A	Kit B	Kit A	Kit B	Alternative method results	(additional) Confirmatory test results by culture	Combined Crono-Salm inoculation number (also see Annex K)	*Salmonella* inoculation (CFU/Sample)
1a	56	1	(PA)	(PA)	ND	ND	-	+	6	5,6
1a	58	1	ND	ND	ND	ND	-	+	8	<1
1c	42	1	ND	ND	ND	ND	-	+	12	<1
2a	156	1	(PA)	(PA)	ND	ND	-	+	6	5,6
2a	157	1	ND	(PA)	ND	ND	-	+	7	3,3
2a	156	2	(PA)	(PA)	ND	ND	-	+	6	5,6
2a	157	2	ND	(PA)	(PA)	(PA)	-	+	7	3,3
3a	31	1	(PA)	(PA)	ND	ND	-	+	1	0,7
3a	39	1	(PA)	(PA)	ND	ND	-	+	9	4,5
3b	224	1	(PA)	(PA)	ND	(PA)	-	+	14	4,1
3c	239	1	(PA)	(PA)	(PA)	ND	-	+	29	3,5
4a	91	1	(PA)	(PA)	ND	ND	-	+	1	0,7
4a	97	1	(PA)	(PA)	ND	(PA)	-	+	7	3,3

Positive deviations per alternative method protocol are listed in Table 11, all concerning sample no. 93.

Table 11 - Positive deviations

			StarPrep One Kit		MagPrep IV Kit					
Category/ Type	Sample n°	Alt method protocol	Kit A	Kit B	Kit A	Kit B	Alternative method results	(additional) Confirmatory test results by culture	Combined Crono-Salm inoculation number (also see Annex K)	*Salmonella* inoculation (CFU/Sample)
4a	93	1	PD	PD	PD	PD	-	+*	3	1,6

* by re-testing 10 ml cold-stored initial BPW using ISO 6579 (MSRV)

For each of the alternative method protocols, the analysis of discordant results according to ISO 16140-2:2016 for a **paired** study is given in Tables 12 (PCR test kit A) and Tables 13 (PCR test kit B).

Table 12-S – Interpretation of the sensitivity study results: StarPrep One Kit, *PCR kit A*

Category	Negative Deviations (ND[1])	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)
1	2	0	2	3	2	6
2.1	1	0	1	3	1	6
2.2	1	0	1	3	1	6
3	0	0	0	3	0	6
4	0	1	-1	3	1	6
All Categories (1, 2.1, 3, 4)	3	1	2	5	4	12
All Categories (1, 2.2, 3, 4)	3	1	2	5	4	12

[1] ND: including PPND

Table 13-S – Interpretation of the sensitivity study results: StarPrep One Kit, *PCR kit B*

Category	Negative Deviations (ND[1])	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)
1	3	0	3	3	3	6
2.1	0	0	0	3	0	6
2.2	0	0	0	3	0	6
3	0	0	0	3	0	6
4	0	1	-1	3	1	6
All Categories (1, 2.1, 3, 4)	3	1	2	5	4	12
All Categories (1, 2.2, 3, 4)	3	1	2	5	4	12

[1] ND: including PPND

Table 12-M – Interpretation of the sensitivity study results: MagPrep IV, *PCR kit A*

Category	Negative Deviations (ND[1])	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)
1	3	0	3	3	3	6
2.1	2	0	2	3	2	6
2.2	1	0	1	3	1	6
3	3	0	3	3	3	6
4	2	1	1	3	3	6
All Categories (1, 2.1, 3, 4)	10	1	9	5	11	12
All Categories (1, 2.2, 3, 4)	9	1	8	5	10	12
Categories 1 and 2.2	4	0	4	4	4	8

[1] ND: including PPND

Table 13-M – Interpretation of the sensitivity study results: MagPrep IV, *PCR kit B*

Category	Negative Deviations (ND[1])	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)
1	3	0	3	3	3	6
2.1	2	0	2	3	2	6
2.2	1	0	1	3	1	6
3	3	0	3	3	3	6
4	1	1	0	3	2	6
All Categories (1, 2.1, 3, 4)	9	1	8	5	10	12
All Categories (1, 2.2, 3, 4)	8	1	7	5	9	12
Categories 1 and 2.2	4	0	4	4	4	8

[1] ND: including PPND

For the alternative method protocol Magnetic Preparation Kit IV *(PCR kit A and PCR kit B*), the observed values for ND-PD and ND+PD for each of the individual categories meet the acceptability limits (observed values ≤ AL). Regarding the combination of "all categories", the observed values for ND+PD also meet the acceptability limit, but the observed value for ND-PD was found to be > AL (Table 12-M and Table 13-M).
In comparison to the StarPrep One Kit results (Table 12-S and Table 13-S), the Magnetic Preparation Kit IV seems to result in more ND results mainly in the Categories 2.1, 3, and 4. After discussion, the MVTC therefore decided (36th meeting) that the validation scope of the Magnetic Preparation Kit IV will be **restricted to the Categories 1 and 2.2 only** (Infant formula and infant cereals, and Probiotics containing products with the specific protocol P2 for sub-cultivation).

3.1.7 Conclusion sensitivity study

The **food**proof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27),

- In combination with the **food**proof® StarPrep One Kit for manual DNA extraction:

The observed values for ND-PD and ND+PD for the 4 individual categories (Infant formula and infant cereals, Probiotics containing products, Ingredients, Environmental samples) and for all categories meet the acceptability limits (observed values ≤ AL).

- In combination with the **food**proof® Magnetic Preparation Kit IV for automated DNA extraction:

The observed values for ND-PD and ND+PD for the restricted 2 individual categories (Infant formula and infant cereals, and Probiotics containing products with the specific protocol P2 for sub-cultivation) and for both categories meet the acceptability limits (observed values ≤ AL).

3.2 Relative level of detection study

The relative level of detection is the level of detection at P = 0,50 (LOD_{50}) of the alternative method divided by the level of detection at P = 0,50 (LOD_{50}) of the reference method.

3.2.1 Categories, sample types and strains

One item and one relevant target micro-organism for this item was chosen for each of the Categories in this validation study, as shown in Table 14.

Table 14 - List of selected items and strains per category, as tested within the relative level of detection study.

Category	Item	Strain	Reference number	Strain origin	Seeding / spiking procedure
Infant formula/ infant cereals	Infant formula	*Salmonella Paratyphi B var. Java*	Salm 5F	Skim milk powder	at least 2 weeks at room temperature
Probiotic containing products	Infant fomula plus probiotics (L. reuteri)	*Salmonella* Typhimurium	ATCC 14028	Chicken organs	Lenticule based
Ingredients	Starch	*Salmonella* Derby	NCTC 5722	Not stated	Lenticule based
Environmental samples	Vacuum cleaner residues	*Salmonella* Stourbridge	S95	Environment	at least 2 weeks at room temperature

3.2.2 Test sample preparations

At least 3 different levels of inoculation per separate target micro-organism and item were tested by both the reference method and the alternative methods:

- Negative control, which was non-inoculated (5x)
- A level producing fractional recovery, which was inoculated with 0,59 – 1 CFU per sample (20x)
- A higher level, which was inoculated with 5,9 – 10 CFU per sample (5x).

Test portions were individually inoculated and kept at an appropriate time/temperature for stabilization before actual testing (see Table 14).

3.2.3 RLOD study results

The tabulated raw data on the RLOD study are given in Annex M.

The RLOD calculations were performed using the Excel spread sheet (version 06-07-2015) as described in ISO 16140-2: 2016.
The RLOD per Category and for each of the alternative method protocols under study is given in Tables 15.

Upon request, also POD-LOD calculations were performed. Results are given in Annex N, for information only.

Table 15-S – Presentation of RLOD before and after confirmation of the alternative method results: StarPrep One Kit, PCR test kit A plus test kit B

Category	Item	Alt method protocol	RLOD using the alternative method results		RLOD using the confirmed alternative method results	
			PCR kit A	PCR kit B	PCR kit A	PCR kit B
1	Infant formula	1	1,000	1,000	1,000	1,000
2.1	Infant formula containing probiotics	1	1,000	1,000	1,000	1,000
2.2	Infant formula containing probiotics	2	1,000	1,000	1,000	1,000
3	Starch	1	1,000	1,000	1,000	1,000
4	Vacuum cleaner residues	1	1,000	1,000	1,000	1,000
Combined			*1,000*	*1,000*	*1,000*	*1,000*

Table 15-S – Presentation of RLOD before and after confirmation of the alternative method results: MagPrep IV, PCR test kit A plus test kit B

Category	Item	Alt method protocol	RLOD using the alternative method results		RLOD using the confirmed alternative method results	
			PCR kit A	PCR kit B	PCR kit A	PCR kit B
1	Infant formula	1	1,000	1,000	1,000	1,000
2.1	Infant formula containing probiotics	1	1,000	1,000	1,000	1,000
2.2	Infant formula containing probiotics	2	0,872	0,872	0,872	0,872
3	Starch	1	1,000	1,000	1,000	1,000
4	Vacuum cleaner residues	1	1,208	1,000	1,208	1,000
Combined			*1,000*	*0,973*	*1,000*	*0,973*

3.2.4 Conclusion RLOD study

The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 1,5 for paired studies, for all 4 Categories and for each of the alternative method protocols as studied.

3.3 Inclusivity and exclusivity study

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.
Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

3.3.1 Protocols inclusivity and exclusivity study

To meet the new criterion in ISO 16140-2:2016 on the number of *Salmonella* strains to be tested for inclusivity (n=100), another 57 strains of *Salmonella* spp. were selected for inclusivity testing, in addition to the 50 strains already tested in the original validation study.

Overnight cultures of pure strains in BPW (18 ± 2h at 37°C) were diluted in peptone saline to contain about 10 -100 cfu/ml and added (1 ml) to fresh portions of 90 ml BPW and processed according to the alternative method only, using the original DNA extraction method (StarPrep One kit). No matrix was added.

3.3.2 Results inclusivity and exclusivity study

All tabulated raw data on inclusivity, both from the original study and the additionally tested strains, are given in Annex O.

The tabulated raw data on exclusivity from the original study are given in Annex P.

A total of 107 *Salmonella* strains were tested for inclusivity. All of these strains showed the expected positive result with the alternative method.
A total of 30 non-*Salmonella* strains were tested for exclusivity. All of these strains showed the expected negative result with the alternative method.

3.3.3 Conclusion inclusivity and exclusivity study

The alternative *Salmonella* detection method protocols as studied are selective and specific.

3.4 Conclusions Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

On the **food**proof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27),

- In combination with the **food**proof® StarPrep One Kit for manual DNA extraction:
    - The observed values for ND-PD and ND+PD for the 4 individual categories (Infant formula and infant cereals, Probiotics containing products, Ingredients, Environmental samples) and for all categories meet the acceptability limits (observed values ≤ AL).
    - The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 1,5 for paired studies, for both categories tested.
    - The alternative *Salmonella* detection method is selective and specific.

- In combination with the **food**proof® Magnetic Preparation Kit IV for automated DNA extraction:
    - The observed values for ND-PD and ND+PD for the restricted 2 individual categories (Infant formula and infant cereals, and Probiotics containing products with the specific protocol P2 for sub-cultivation) and for both categories meet the acceptability limits (observed values ≤ AL).
    - The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 1,5 for paired studies, for both categories tested.
    - The alternative *Salmonella* detection method is selective and specific.

4. Interlaboratory Study

The inter-laboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

4.1 Study organisation

The interlaboratory study data as obtained during the original LR8/9/19/20 validation studies are considered to be valid as well for the more recent extension and renewal studies.
All detailed information on the organization of the interlaboratory study, sample preparations and distribution, controls, etc., as well as all raw data on the interlaboratory study can be found in the original 2011LR39 ILS report (RIVM Confidential Report 330634002/2012).
The existing interlaboratory study data were successfully re-evaluated according to the ISO 16140-2 (2016) and the MVTC interpretation document (doc 2016-082 GC) and described in the Renewal report (RIVM-Z&O Confidential Letter-Report 00109/2017).

4.2 Calculation and summary of data

The overview on the ILS results from the expert laboratory (EL) and from all 15 collaborative laboratories (A-N) is given in Annex R.

4.3. Results of the collaborators retained for interpretation

Laboratories H and L reported several positive results for some of the blank samples, but they also mentioned leakage or overflow of the sample bags during analysis. The data sets from laboratories H and L were therefore excluded from the statistical evaluation of the study.
According to the MVTC interpretation of ISO 16140-2 (doc 2016-028 (GC)) all data from collaborators that found (false) positive results with the blank samples, either by the reference or alternative method, were disregarded for the renewal study as well.
The results obtained with the 11 collaborators kept for interpretation are presented in Table 16 (reference method) and Table 17 (alternative method).

Table 16 - Positive results by the reference method (without collaborators H, I, L, M)

	Contamination Level		
Collaborator	L0	L1	L2
A	0	7	8
B	0	7	8
C	0	7	8
D	0	8	8
E	0	7	8
F	0	8	8
G	0	8	8
J	0	8	8
K	0	8	8
N	0	8	8
O	0	8	8
Total	**0**	**84**	**88**
	P0	P1	P2

Table 17 - Positive results (before and after confirmation) by the alternative method (without collaborators H, I, L, M)

Collaborator	Contamination Level					
	L0		L1		L2	
	Before confirmation	After confirmation	Before confirmation	After confirmation	Before confirmation	After confirmation
A	0	0	7	7	8	8
B	0	0	7	7	8	8
C	0	0	7	7	8	8
D	0	0	8	8	8	8
E	0	0	7	7	8	8
F	0	0	8	8	8	8
G	0	0	8	8	8	8
J	0	0	8	8	8	8
K	0	0	8	8	8	8
N	0	0	8	8	8	8
O	0	0	8	8	8	8
Total	**0**	**0**	**84**	**84**	**88**	**88**
	P0	**CP0**	**P1**	**CP1**	**P2**	**CP2**

4.4. Calculation of the specificity (SP)

The percentage specificity (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 is given in Table 18.

Table 18 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	100 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	100 %

N_- number of all L0 tests
P_0 total number of false-positive results obtained with the blank samples, for the reference method
CP_0 total number of false-positive results obtained with the blank samples, for the alternative method after confirmation

4.5. Calculation of the sensitivity (SEalt, SEref), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low inoculation level (L1) only. This inoculation level was retained for calculations.
A summary of the results of the collaborators retained for interpretation, and obtained with the reference and the alternative methods for Level 1 is provided in Table 19.

Table 19 - Summary of the obtained results with the reference method and the alternative method for Level 1

Response	**Reference method positive (R+)**	**Reference method negative (R-)**
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 84**	Positive deviation (R-/A+) **PD = 0**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 4**

Based on the data summarized in Table 19, the values of sensitivity of the alternative and reference methods, as well as the relative trueness and false positive ratio for the alternative method taking account the confirmations, are given in Table 20.

Table 20 - Sensitivity, relative trueness and false positive ratio percentages

		Level 1
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} x\ 100\% =$	100%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} x\ 100\% =$	100%
Relative trueness	$RT = \frac{(PA+NA)}{N} x\ 100\% =$	100%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} x\ 100\% =$	0%

4.6. Interpretation of data

No negative deviations or positive deviations were seen in the results of the 11 collaborators retained for interpretation.

For a paired study design, the difference between (ND – PD) and the addition (ND + PD) are calculated for the level(s) where fractional recovery is obtained (in this study L1 only). The observed value found for (ND – PD) and (ND + PD) shall not be higher than the AL.
The interpretation of the data (11 collaborators) is given in Table 21.

Table 21 – Interpretation of the interlaboratory study results

Level	Negative Deviations (ND[1])	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)
L1	0	0	0	4	0	4

[1] ND including PPND

4.7. Conclusion Interlaboratory Study

The observed values for ND-PD and ND+PD (inoculation level L1, 11 collaborators) meet the acceptability limits (observed values ≤ AL).

4.8. Evaluation of the RLOD between laboratories

The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at http://standards.iso.org/iso/16140 - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 06.07.2015). The results are for information only (see Table 22).

Table 22 – RLOD calculations interlaboratory study data 11 laboratories

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statist	p-value
all 11 labs, 3 levels	1,000	0,661	1,512	0,000	0,207	0,000	1,000

5. Overall Conclusions Validation Study

The conclusions for the **Method Comparison Study** are:

On the **food**proof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27),

- In combination with the **food**proof® StarPrep One Kit for manual DNA extraction:
  - The observed values for ND-PD and ND+PD for the 4 individual categories (Infant formula and infant cereals, Probiotics containing products, Ingredients, Environmental samples) and for all categories meet the acceptability limits (observed values ≤ AL).
  - The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 1,5 for paired studies, for both categories tested.
  - The alternative *Salmonella* detection method is selective and specific.

- In combination with the **food**proof® Magnetic Preparation Kit IV for automated DNA extraction:
  - The observed values for ND-PD and ND+PD for the restricted 2 individual categories (Infant formula and infant cereals, and Probiotics containing products with the specific protocol P2 for sub-cultivation) and for both categories meet the acceptability limits (observed values ≤ AL).
  - The RLOD values (using the confirmed alternative method results) meet the acceptability limit, which is 1,5 for paired studies, for both categories tested.
  - The alternative *Salmonella* detection method is selective and specific.

The conclusion for the **Interlaboratory Study** is:

- The observed values for ND-PD and ND+PD (inoculation level L1, 11 collaborators) meet the acceptability limits (observed values ≤ AL).

The overall conclusions of the validation study are:

The alternative method **food**proof® *Salmonella* Detection Kit (Hybridization probes and 5'Nuclease), using the **food**proof® StarPrep One Kit for manual DNA extraction, shows comparable performance to the reference method ISO 6579-1:2017 for the detection of *Salmonella* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method **food**proof® *Salmonella* Detection Kit (Hybridization probes and 5'Nuclease), using the **food**proof® Magnetic Preparation Kit IV for automated DNA extraction, shows comparable performance to the reference method ISO 6579-1:2017 for the detection of *Salmonella* in Infant formula and infant cereals, and in Probiotics containing products.

The scope for the **food**proof® Magnetic Preparation Kit IV for automated DNA extraction is **restricted** to Infant formula and infant cereals, and Probiotics containing products, the latter needing the specific sub-cultivation protocol (second BPW for 20 h at 37°C).

The overview on the claims for the current LR39 is given in the Table below:

Detection of *Salmonella*

	Enrichment	Standard protocol: 100 g sample material* plus 900 ml BPW pre-warmed at 37 °C, 16 - 20 h at 37 °C ± 1 °C									
	Subcultivation	Standard protocol P1: 100 µl of BPW culture in 900 µl BPW, 3 - 4 h at 37 °C ±1 °C (shaking at 900 U/min)									
		Specific protocol P2: 100 µl of BPW culture in 900 µl BPW, 20 - 24 h at 37 °C ± 1 °C (shaking at 900 U/min)									
	Reagent D treatment	A 500 02									
	DNA extraction	Manual protocol **food**proof StarPrep One Kit					Automated protocol **food**proof MagPrep IV Kit				
		S 400 07					S 400 15				
	Real-time PCR, pre-screening on EB	**food**proof EBC detection kit, Hybridization probes		**food**proof EBC detection kit, 5'Nuclease			**food**proof EBC detection kit, Hybridization probes		**food**proof EBC detection kit, 5'Nuclease		
		R 310 15.1		R 302 15.1			R 310 15.1		R 302 15.1		
	Real-time PCR, *Salmonella* detection	**food**proof S detection kit, Hybridization probes		**food**proof S detection kit, 5'Nuclease			**food**proof S detection kit, Hybridization probes		**food**proof S detection kit, 5'Nuclease		
		R 310 27		R 302 27			R 310 27		R 302 27		
	Thermocyclers	LC 480 II	LC 2.0	LC 480 II	IQ5	Mx 3005	LC 480 II	LC 2.0	LC 480 II	IQ5	Mx 3005
	Software version	1.5.1	4.1	1.5.1	2.0	MX4.1d	1.5.1	4.1	1.5.1	2.0	MX4.1d
Scope (including subcultivation Protocol)	Infant formula and infant cereals (P1)	X	X	X	X	X	X	X	X	X	X
	Probiotics containing products (P1)	X	X	X	X	X					
	Probiotics containing products (P2)	X	X	X	X	X	X	X	X	X	X
	Ingredients (P1)	X	X	X	X	X					
	Environmental samples (P1)	X	X	X	X	X					

*specific sample preparations: see Annex C or EBC detection kit inserts

References

ISO 4833-1:2013; Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms -- Part 1: Colony count at 30 degrees C by the pour plate technique

ISO 6887; Microbiology of food and animal feeding stuffs -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – All parts.

ISO 6579:2002; Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

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.

ISO 6887-4:2017; Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 4: Specific rules for the preparation of miscellaneous products.

ISO 7218; Microbiology of food and animal feeding stuffs -- General requirements and guidance for microbiological examinations.

ISO 16140-2:2016; Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

ISO 17468; Microbiology of the food chain -- Technical requirements and guidance on establishment or revision of a standardized reference method.

ISO 27205:2010 (IDF 149:2010); Fermented milk products -- Bacterial starter cultures -- Standard of identity.

Jacobs-Reitsma, W.F., E.M. Bouw, W.M. van Overbeek, and M. Wuite. **Method Comparison Study Report MicroVal project 2011LR39**. ISO 16140:2003 Validation of the **food**proof® *Enterobacteriaceae* plus *E. sakazakii* Detection Kit, followed by the **food**proof® *Salmonella* Detection Kit for the detection of *Salmonella* in powdered infant formula, probiotic culture powders, and ingredients. RIVM Confidential Report 330634001/2012.

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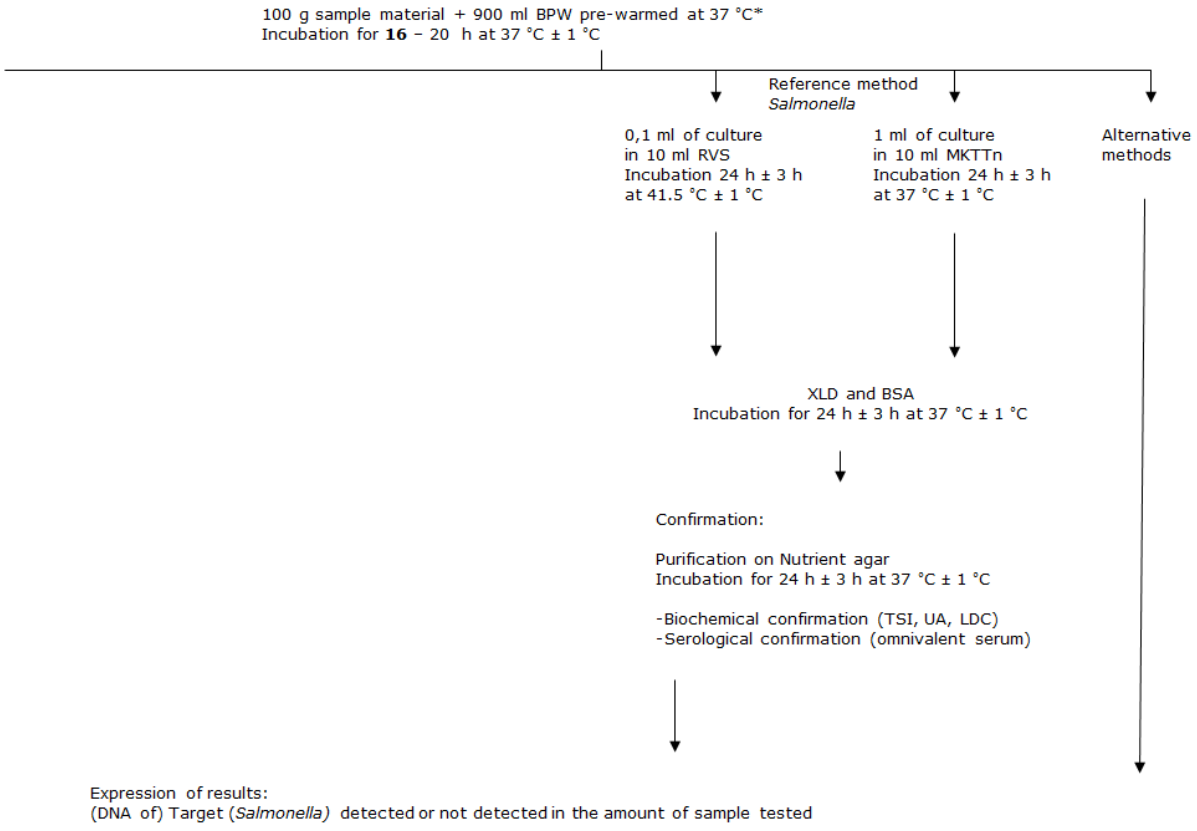
Jacobs-Reitsma, W.F., R. Diddens, and E.M. Bouw. **Renewal Report for MicroVal project 2011LR39**, concerning the **food**proof® *Salmonella* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27) for detection of *Salmonella* in powdered infant formula, probiotic culture powders, and ingredients. RIVM-Z&O Confidential Letter-Report 00109/2017.

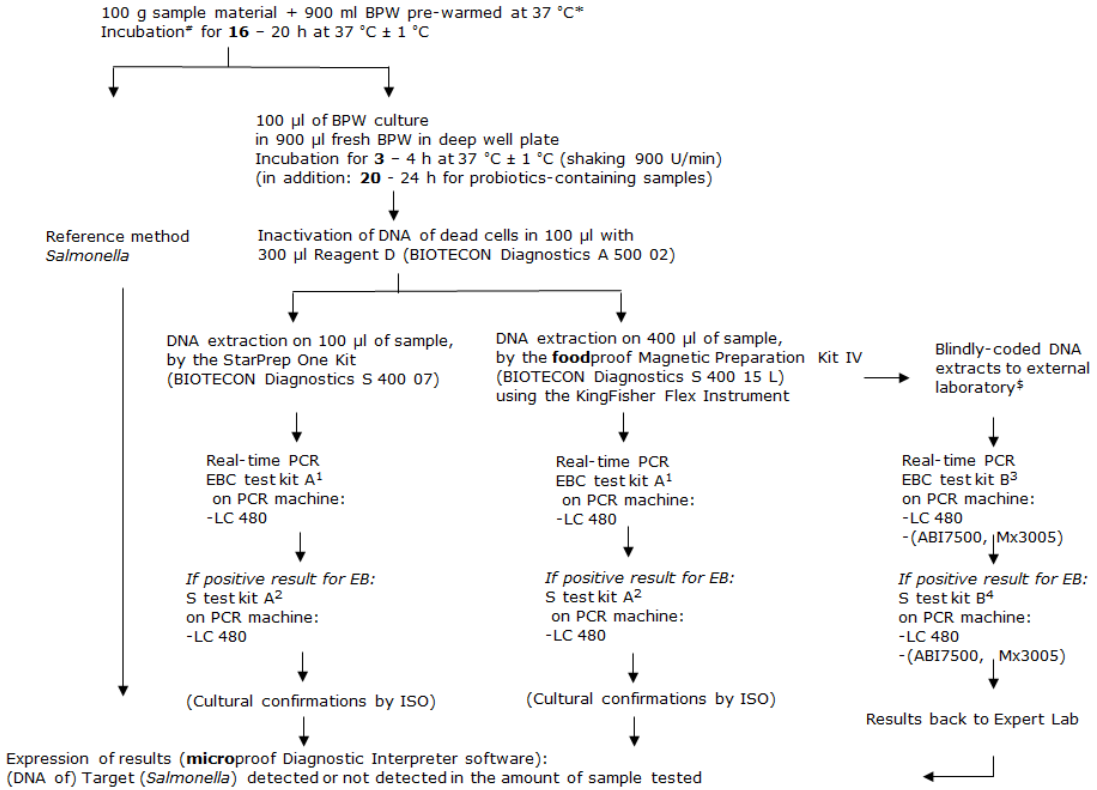
Annex A. Diagram of the reference method (ISO 6579:2002 / ISO 6579:2017)



*Some sample types are tested according to an adjusted pre-enrichment protocol, see Introduction and Annex C.

All incubations are done according to the *minimum* incubation time (indicated in bold).
For confirmation: Test one Target-suspect colony. If negative, test up to 4 additional suspect colonies

Annex B. Diagram of alternative methods



*Some sample types are tested according to an adjusted pre-enrichment protocol, see Introduction and Annex C.
All incubations are done according to the *minimum* incubation time (indicated in bold).
$ Overnight delivery on dry-ice of DNA extracts in microfuge tubes.
[1] EBC test kit A: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - Hybridization probes - (BIOTECON Diagnostics R 310 15)
[2] S test kit A: **food**proof® *Salmonella* Detection Kit - Hybridization probes - (BIOTECON Diagnostics R 310 27)
[3] EBC test kit B: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - 5'Nuclease - (BIOTECON Diagnostics R 302 15)
[4] S test kit B: **food**proof® *Salmonella* Detection Kit – 5'Nuclease - (BIOTECON Diagnostics R 302 27)

Annex C. Sample preparations and enrichment protocols

The following tables show the sample preparation and pre-enrichment protocols for the 4 Categories and their Types of samples.

General remarks:
BPW, pre-heated at 37°C, at the time of sample preparation.
All samples: 100 gram samples, unless otherwise stated.
Unless otherwise stated sample preparations are according to ISO 6887-5 for the dry infant formula products. This reads: In order to dissolve the sample, swirl slowly to wet the powder, then manually shake the bottle, e.g. 25 times, with a movement of about 300 mm, for about 7 s. Alternatively, a peristaltic blender may be used. Allow to stand for 5 min, shaking occasionally.

Table C-1 Category: Infant Formula and infant cereals.

Sample Type	Sample preparation	Pre-enrichment
Infant Formula (intended for infants < 1 year)	BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C
Infant Formula (intended for infants > 1 year)	BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C
Infant cereals	BPW + alpha-amylase (alpha-amylase at 50 mg per 100 gram sample in 900 ml BPW for products with high starch content)	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C

Table C-2 Category: Probiotics containing products.

Sample Type	Sample preparation	Pre-enrichment
Infant formula containing probiotics (*L. paracasei, L. rhamnosis, L. reuteri*) at a level < 10^8 cfu/g (consumer products)	BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h and 20 h at 37°C ± 1°C
Infant formula containing probiotics (*L. johnsonii, S. thermophilus, B. lactis, B. longum*) at a level < 10^8 cfu/g (consumer products)	BPW plus vancomycin (vancomycin at 10 mg/l)	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h and 20 h at 37°C ± 1°C
Infant cereals containing probiotics (*Bifidus* bacteria) at a level < 10^8 cfu/g (consumer products)	BPW plus vancomycin and alpha-amylase (vancomycin at 10 mg/l) (alpha-amylase at 50 mg per 100 gram sample in 900 ml BPW for products with high starch content)	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h and 20 h at 37°C ± 1°C
Culture powders / Preblends containing *L. reuteri* at ~10^{10} cfu/g	double strength BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h and 20 h at 37°C ± 1°C
Culture powders / Preblends containing *L. rhamnosis* and *B. longum* at ~10^{10} cfu/g	double strength BPW plus vancomycin (vancomycin at 10 mg/l)	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h and 20 h at 37°C ± 1°C

Table C-3 Category: Ingredients.

Sample Type	Sample preparation	Pre-enrichment
Standard PIF ingredients (e.g. milk cow powder, whey cow powder, lactose, maltodextrine)	BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C
Infant cereals ingredients (e.g. starch, oatmeal, rye meal, wheat(flour), buckwheat)	BPW plus alpha-amylase (alpha-amylase at 50 mg per 100 gram sample in 900 ml BPW for products with high starch content)	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C
Premix, Duomix (containing minerals, vitamins)	12,5 gram in 900 BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C

Table C-4 Category: Environmental samples.

Sample Type	Sample preparation	Pre-enrichment
Sweep samples/equipment swabs	Submerge swab/sponge in 90 ml BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C
Traject samples (in-line factory)	BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C
Vacuum cleaner residues	BPW	Incubation 18 h ±2 h at 37°C ± 1°C, followed by 100 µl cultured pre-enrichment in 900 µl fresh BPW Incubation (shaking) 3 h at 37°C ± 1°C

The following abbreviations are used (e.g. also in Annex L on the raw sensitivity data) to indicate the various different sample preparations:

AA	Alpha-amylase added to initial BPW (at 50 mg per 100 gram sample)
BV	Vancomycin added to initial BPW (at 10 mg/l)
D	Double-concentrated BPW
DV	Double-concentrated BPW plus vancomycin (at 10 mg/l)
12,5	12,5 gram sample in 900 ml BPW
90	90 ml BPW (swab/sponge-type samples)

Annex D-J. Kit Inserts

For practical reasons, Kit inserts will be only available as separate pdf's.
All kit inserts will also be available on the website of BIOTECON Diagnostics:
www.bc-diagnostics.com

Annex D*: Kit insert reagent D (BIOTECON Diagnostics Cat. No. A 500 02; version 3, April 2018).

Annex E*: Kit insert **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07; version 6, November 2018).

Annex F*: Kit insert **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15; version 1, August 2014).

Annex G*: Kit insert EBES test kit A: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - Hybridization probes - (BIOTECON Diagnostics Cat. No. R 310 15.1; version 5, October 2018, as updated according to MicroVal rules).

Annex H*: Kit insert EBES test kit B: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - 5'Nuclease - (BIOTECON Diagnostics Cat. No. R 302 15.1; version 3, November 2018, as updated according to MicroVal rules).

Annex I: Kit insert S test kit A: **food**proof® *Salmonella* Detection Kit - Hybridization probes - (BIOTECON Diagnostics Cat. No. R 310 27; version 5, October 2018, as updated according to MicroVal rules).

Annex J: Kit insert S test kit B: **food**proof® *Salmonella* Detection Kit – 5'Nuclease - (BIOTECON Diagnostics Cat. No. R 302 27; version 5, November 2018, as updated according to MicroVal rules).

*: Identical Annex for both the LR 39 and the LR 8/9/19/20 studies.

Annex K. Artificial inoculation of test samples

C + S Combination nr. (Annex L)	Cronobacter strain	Strain code	Origin	Source	inoculation (cfu/sample)	Salmonella serovar	Strain code	Origin	Source	inoculation (cfu/sample)
1	*Cronobacter sakazakii*	4485 D	DSM 4485 (WDCM 00214)	Child's throat	2,5	Agona	MV1-F		Milk powder	0,7
2	*Cronobacter turicensis*	18703 D	DSM 18703	Human neonate	1,5	Livingstone	MV2-F		Horsemilk powder	2,2
3	*Cronobacter dublinensis*	18705 D	DSM 18705	Milk powder facility	2,4	Manhattan	MV3-F		Powder product	1,6
4	*Cronobacter muytjensii*	21870 D	DSM 21870 (WDCM 00213)	Unknown	6,4	Oranienburg	MV4-F		Milk powder	3,4
5	*Cronobacter sakazakii*	Micr 488	Europe	Infant formula	<1	Paratyphi B - Java	MV5-F		Skim milk powder	0,4
6	*Cronobacter sakazakii*	Micr 493	Europe	Milk pudding	1,2	Isangi	MV6-F		Skim milk powder	5,6
7	*Cronobacter sakazakii*	Micr 507	Europe	Cereals	2,9	Montevideo	MV7-F		Milk powder	3,3
8	*Cronobacter sakazakii*	Micr 510	Europe	Follow-up formula	2,8	Senftenberg	MV10-H		Milk powder	<1
9	*Cronobacter sakazakii*	Micr 518	USA	Environment	4,3	Cubana	MV11-F		Milk powder	4,5
10	*Cronobacter sakazakii*	Micr 489	Europe	Follow-up formula	3,6	Enteritidis PT 3	MV13-F		Milk powder	<1
11	*Cronobacter sakazakii*	Micr 508	Europe	Follow-up formula	1,8	Livingstone	Salm 261-F		Infant formula	<1
12	*Cronobacter sakazakii*	Micr 561	Switzerland	Environment: sponge	7,1	Infantis	Salm 691-D		Milk powder	<1
13	*Cronobacter sakazakii*	Micr 562	Switzerland	Water: fountain	2,2	Senftenberg	719-D		cocoa	<1
14	*Cronobacter sakazakii*	Micr 509	Europe	Infant formula	<1	Babelsberg	S53	United Kingdom	Foods	4,1
15	*Cronobacter sakazakii*	Micr 568	Australia	Milk powder	<1	Bangkok	S54	Thailand	Foods	1,5
16	*Cronobacter sakazakii*	Micr 569	Canada	Milk powder	<1	Blockley	S56		Meat products	5,4
17	*Cronobacter sakazakii*	Micr 570	Russia	Milk powder	4,9	Bochum	S57		Environment	<1
18	*Cronobacter sakazakii*	Micr 574	Indonesia	Milk powder	0,2	Braenderup	S60		Foods	<1
19	*Cronobacter sakazakii*	Micr 576	Switzerland	Fruit powder	4,4	Brandenburg	S61	Spain	Foods	<1
20	*Cronobacter sakazakii*	Micr 579	Canada	Environment	<1	Canstatt	S62		Animal feed	1,7
21	*Cronobacter sakazakii*	Micr 580	Uruguay	Milk powder	<1	Frankfurt	S69	Nigeria	Foods	<1
22	*Cronobacter sakazakii*	Micr 581	New Zealand	Infant formula	0,9	Isangi	S71		Bone meal	2,7
23	*Cronobacter sakazakii*	Micr 582	USA	Rice flour	1,6	Kaapstad	S72	Italy	Foods	3,3
24	*Cronobacter malonaticus*	Micr 595	DSM 18702T, Type strain	Breast abces	<1	Krefeld	S76		Animal feed	<1
25	*Cronobacter dublinensis*	Micr 596	DSM 18706T, Type strain	Water fountain basin	3,2	Langenhorn	S77		Spices	3,7
26	*Cronobacter sakazakii*	Micr 374	France	Infant formula	3,8	Langford	S78	Spain	Foods	3,6
27	*Cronobacter muytjensii*	Micr 485	ATCC 51329, Type strain	Not stated	1,9	Liverpool	S79	Venezuela	Foods	4,2
28	*Cronobacter sakazakii*	Micr 486	Europe	Environment	1,7	Llandoff	S80		Foods	4,8
29	*Cronobacter sakazakii*	Micr 496	Europe	Environment	0,8	Napoli	S83	Italy	Foods	3,5
30	*Cronobacter sakazakii*	Micr 497	Europe	Environment	4,3	Orion	S85		Bone meal	<1
31	*Cronobacter sakazakii*	Micr 489	Europe	Follow-up formula	3,9	Plymouth	S88		Others	3,9
32	*Cronobacter sakazakii*	Micr 508	Europe	Follow-up formula	6,7	Putten	S90		Bone meal	2,5
33	*Cronobacter sakazakii*	Micr 561	Switzerland	Environment: sponge	2,9	Ramatgan	S91	Singapore	Foods	1,3
34	*Cronobacter sakazakii*	Micr 562	Switzerland	Water: fountain	1,8	Sandiego	S93	Mexico	Foods	1,6
35	*Cronobacter sakazakii*	Micr 510	Europe	Follow-up formula	2,4	Stourbridge	S95		Environment	1,6
36	*Cronobacter sakazakii*	Micr 518	USA	Environment	1,7	Taksony	S96		Others	1
37	*Cronobacter sakazakii*	Micr 493	Europe	Milk pudding	0,8	Livingstone	MV2-F		Horsemilk powder	<1
38	*Cronobacter sakazakii*	Micr 507	Europe	Cereals	1,4	Babelsberg	S53	United Kingdom	Foods	<1
39	*Cronobacter sakazakii*	Micr 576	Switzerland	Fruit powder	2,3	Bangkok	S54	Thailand	Foods	3,6
40	*Cronobacter sakazakii*	Micr 580	Uruguay	Milk powder	2,9	Blockley	S56		Meat products	4
41	*Cronobacter muytjensii*	Micr 485	ATCC 51329, Type strain	Not stated	4,6	Langenhorn	S77		Spices	2,8
42	*Cronobacter sakazakii*	Micr 486	Europe	Environment	<1	Napoli	S83	Italy	Foods	4
43	*Cronobacter sakazakii*	Micr 497	Europe	Environment	1,5	Manhattan	MV3-F		Powder product	<1
44	*Cronobacter sakazakii*	4485 D	DSM 4485 (WDCM 00214)	Child's throat	<1	Isangi	MV6-F		Skim milk powder	0,4
45	*Cronobacter sakazakii*	Micr 509	Europe	Infant formula	<1	Montevideo	MV7-F		Milk powder	<1
46	*Cronobacter sakazakii*	Micr 568	Australia	Milk powder	0,9	Brandenburg	S61	Spain	Foods	4,2
48	*Cronobacter malonaticus*	Micr 595	DSM 18702T, Type strain	Breast abces	3,3	Frankfurt	S69	Nigeria	Foods	1,9
Salm only (S97)						S. Thompson	S97		Animal feed	5,6
Salm only (S99)						S. Typhimurium	S99	1,4,[5],12:i:-	Human	4,5

Annex L. Raw data sensitivity study

All raw data on the sensitivity study are compiled in a separate excel file. Details are available upon request.

Annex M. Tabulated raw data RLOD study

StarPrep One Kit, Category 1.

Matrix: **Infant formula** **Inoculation strain:** ***S.* Paratyphi B var Java, originating from skim milk powder (MV 5-F)**

		Reference methods									StarPrep One Kit					StarPrep One Kit				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
532.1	0	ng	-	ng	ng	ng	ng	-	0/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
532.2		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.3		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.4		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.5		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.36	1	ng	-	ng	ng	ng	ng	-	17/20	17/20	neg	neg	neg	17/20	17/20	neg	neg		17/20	17/20
532.40		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.42		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.31		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.32		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.34		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.37		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.38		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.41		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.43		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.45		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.46		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.50		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.51	10	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
532.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex M. Tabulated raw data RLOD study, continued

StarPrep One Kit, Category 2.1

Matrix: **Probiotic Infant formula (L.reuteri)** **Inoculation strain:** **S. Typhimurium (lenticule)**

		Reference methods									StarPrep One Kit					StarPrep One Kit				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
544.1	0	ng	-	ng	ng	ng	ng	-	0/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
544.2		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.3		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.4		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.5		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.37	0,7	ng	-	ng	ng	ng	ng	-	13/20	13/20	neg	neg	neg	13/20	13/20	neg	neg		13/20	13/20
544.40		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.41		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.43		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.45		ng	-	ng	ng	ng	ng	-			rep(*neg)	neg	neg			neg	neg			
544.50		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.42		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.31		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.32		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.34		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.36		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.38		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.46		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.51	7	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
544.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex M. Tabulated raw data RLOD study, continued

StarPrep One Kit, Category 2.2

Matrix: **Probiotic Infant formula (L.reuteri)** **Inoculation strain:** **S. Typhimurium (lenticule)**

		Reference methods									StarPrep One Kit			20 h 2nd BPW		StarPrep One Kit			20 h 2nd BPW	
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary * EB	Data summary * Salmo	PCR kit B			Data summary * EB	Data summary * Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
544.1	0	ng	-	ng	ng	ng	ng	-	0/5	0/5	neg	neg	(rep)	0/5	0/5	neg	neg		0/5	0/5
544.2		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.3		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.4		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.5		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.37	0,7	ng	-	ng	ng	ng	ng	-	13/20	13/20	neg	neg	neg	13/20	13/20	neg	neg		13/20	13/20
544.40		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.41		ng	-	ng	ng	ng	ng	-			rep(*neg)	neg	rep(*neg)			neg	neg			
544.43		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.45		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.50		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.42		ng	-	ng	ng	ng	ng	-			neg	neg	(rep)			neg	neg			
544.31		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.32		S	+	S	S	S	S	+			pos	pos	pos			pos	neg	pos		
544.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.34		S	+	S	S	S	S	+			pos	pos	pos			pos	neg	pos		
544.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.36		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.38		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.39		S	+	S	S	S	S	+			pos	pos	pos			pos	neg	pos		
544.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.46		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.51	7	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
544.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex M. Tabulated raw data RLOD study, continued

StarPrep One Kit, **Category 3.**

Matrix: **Starch** **Inoculation strain:** **S. Derby (lenticule)**

		Reference methods								StarPrep One Kit					StarPrep One Kit				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
702.1	0	ng	-	ng	ng	ng	ng	-	2/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
702.2		S?	+*	ng	ng	ng	ng	-			rep/ neg	neg	neg			neg	neg			
702.3		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.4		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.5		S?	+*	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.34	0,59	ns	-	ng	ng	ng	ng	-	15/20	14/20	neg	neg	neg	14/20	14/20	neg	neg		14/20	14/20
702.38		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.41		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.42		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.46		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.31		S?	+*	ng	ng	ng	ng	-			neg	neg	neg			rep	rep	rep		
702.32		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.33		S	+	S	S	S	S	+			pos	neg	pos			pos	rep	pos		
702.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.36		S	+	S	S	S	S	+			pos	rep/neg	pos			pos	rep	pos		
702.37		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.40		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.43		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.45		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.50		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.51	5,9	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	pos	pos	5/5	5/5
702.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)
*Pantoa spp. naturally present

Annex M. Tabulated raw data RLOD study, continued

StarPrep One Kit, **Category 4.**

Matrix: **Vacuum cleaner residues** **Inoculation strain:** ***S. Stourbridge*** **(S95)**

		Reference methods									StarPrep One Kit					StarPrep One Kit				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
730.1	0	S	+	ns	ns	ns	ns	-	5/5	0/5	pos	pos	neg	5/5	0/5	pos	pos	rep/neg	5/5	0/5
730.2		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.3		S	+	ns	ns	ns	ns	-			pos	neg	neg			pos	pos	rep/neg		
730.4		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	pos/neg		
730.5		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	rep/rep		
730.7	0,7	S	+	ns	ns	ns	ns	-	20/20	7/20	pos	pos	neg	20/20	7/20	pos	pos	neg	20/20	7/20
730.10		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.11		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	rep	neg		
730.14		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	rep	rep/neg		
730.15		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.17		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.18		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.19		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.20		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.21		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	rep	rep/neg		
730.22		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.24		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	rep	rep/neg		
730.25		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	rep	neg		
730.6		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	rep(*pos)			pos	pos	pos		
730.8		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	pos			pos	rep	pos		
730.9		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	pos			pos	rep	pos		
730.12		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.13		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.16		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.23		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.26	7	S	+	S+ns	S+ns	S+ns	S+ns	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
730.27		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.28		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.29		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.30		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex M - Tabulated raw data RLOD study, continued

MagPrep IV, Category 1.

Matrix: **Infant formula** **Inoculation strain:** **S. Paratyphi B var Java (MV 5-F)**

		Reference methods									Magn Prep Kit IV					Magn Prep Kit IV				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
532.1	0	ng	-	ng	ng	ng	ng	-	0/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
532.2		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.3		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.4		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.5		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.36	1	ng	-	ng	ng	ng	ng	-	17/20	17/20	neg	neg	ep (*neg	17/20	17/20	neg	neg		17/20	17/20
532.40		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.42		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
532.31		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.32		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.34		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.37		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.38		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.41		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.43		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.45		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.46		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.50		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.51	10	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
532.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
532.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex M. Tabulated raw data RLOD study, continued

MagPrep IV, Category 2.1

Matrix: **Probiotic Infant formula (L.reuteri)** **Inoculation strain:** **S. Typhimurium (lenticule)**

		Reference methods									Magn Prep Kit IV					Magn Prep Kit IV				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
544.1	0	ng	-	ng	ng	ng	ng	-	0/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
544.2		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.3		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.4		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.5		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.37	0,7	ng	-	ng	ng	ng	ng	-	13/20	13/20	neg	neg	neg	13/20	13/20	neg	neg		13/20	13/20
544.40		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.41		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.43		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.45		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.50		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.42		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.31		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.32		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.34		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.36		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.38		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.46		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.51	7	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
544.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex M. Tabulated raw data RLOD study, continued

MagPrep IV, Category 2.2

Matrix: **Probiotic Infant formula (L.reuteri)** **Inoculation strain:** **S. Typhimurium (lenticule)**

		Reference methods									Magn Prep Kit IV			20 h 2nd BPW		Magn Prep Kit IV			20 h 2nd BPW	
											PCR kit A					PCR kit B				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	EB	Crono	Salm	Data summary * EB	Data summary * Salmo	EB	Crono	Salm	Data summary * EB	Data summary * Salmo
				XLD	BSA	XLD	BSA													
544.1	0	ng	-	ng	ng	ng	ng	-	0/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
544.2		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.3		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.4		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.5		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.37	0,7	ng	-	ng	ng	ng	ng	-	13/20	13/20	neg	neg	neg	14/20	14/20	neg	neg		14/20	14/20
544.40		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.41		ng	-	ng	ng	ng	ng	-			neg	neg	neg			inh/neg	inh/neg			
544.43		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.45		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.50		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
544.42		ng	-	ng	ng	ng	ng	-			pos[1]	neg	pos[1]			pos[1]	neg	pos[1]		
544.31		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.32		S	+	S	S	S	S	+			pos	neg	inh/pos			pos	neg	pos		
544.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.34		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.36		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	neg/pos		
544.38		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.46		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.51	7	S	+	S	S	S	S	+	5/5	5/5	pos	neg	pos	5/5	5/5	pos	neg	pos	5/5	5/5
544.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.53		S	+	S	S	S	S	+			pos	neg	rep(*pos)			pos	neg	pos		
544.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
544.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

[1]: culture-confirmed by re-enrichment of original BPW (MSRV)

Annex M. Tabulated raw data RLOD study, continued

MagPrep IV, Category 3.

Matrix: **Starch** **Inoculation strain:** **S. Derby (lenticule)**

		Reference methods									Magn Prep Kit IV					Magn Prep Kit IV				
				RVS		MKTTn					PCR kit A					PCR kit B				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	XLD	BSA	XLD	BSA	Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	EB	Crono	Salm	Data summary* EB	Data summary* Salmo	EB	Crono	Salm	Data summary* EB	Data summary* Salmo
702.1	0	ng	-	ng	ng	ng	ng	-	2/5	0/5	neg	neg	neg	0/5	0/5	neg	neg		0/5	0/5
702.2		S?	+*	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.3		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.4		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.5		S?	+*	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.34	0,59	ns	-	ng	ng	ng	ng	-	15/20	14/20	neg	neg	neg	14/20	14/20	neg	neg		14/20	14/20
702.38		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.41		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.42		ng	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.46		ns	-	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.31		S?	+*	ng	ng	ng	ng	-			neg	neg	neg			neg	neg			
702.32		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.33		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.35		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.36		S	+	S	S	S	S	+			pos	neg	pos			pos	rep	pos		
702.37		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.39		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.40		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.43		S	+	S	S	S	S	+			rep/ pos	neg	pos			pos	neg	pos		
702.44		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.45		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.47		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.48		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.49		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.50		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.51	5,9	S	+	S	S	S	S	+	5/5	5/5	pos	rep/ pos	pos	5/5	5/5	pos	pos	pos	5/5	5/5
702.52		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.53		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.54		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		
702.55		S	+	S	S	S	S	+			pos	neg	pos			pos	neg	pos		

Data summary: number of positive samples/total number of samples (per level)

*Pantoa spp. naturally present

Annex M. Tabulated raw data RLOD study, continued

MagPrep IV, Category 4.

Matrix: **Vacuum cleaner residues** **Inoculation strain:** ***S. Stourbridge* (S95)**

		Reference methods								Magn Prep Kit IV					Magn Prep Kit IV				
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	RVS		MKTTn		Final confirmed result Salmo	Data summary* EB	Data summary* Salmo	PCR kit A			Data summary* EB	Data summary* Salmo	PCR kit B			Data summary* EB	Data summary* Salmo
				XLD	BSA	XLD	BSA				EB	Crono	Salm			EB	Crono	Salm		
730.1	0	S	+	ns	ns	ns	ns	-	5/5	0/5	pos	pos	inh/neg	5/5	0/5	pos	weak pos	neg	5/5	0/5
730.2		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.3		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	weak pos	neg		
730.4		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.5		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.7	0,7	S	+	ns	ns	ns	ns	-	20/20	7/20	pos	pos	neg	20/20	6/20	pos	pos	neg	20/20	7/20
730.10		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.11		S	+	ns	ns	ns	ns	-			pos	neg	neg			pos	neg	neg		
730.14		S	+	ns	ns	ns	ns	-			pos	neg	neg			pos	neg	neg		
730.15		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.17		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.18		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.19		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	pos	neg		
730.20		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	pos	neg		
730.21		S	+	ns	ns	ns	ns	-			pos	neg	neg			pos	neg	neg		
730.22		S	+	ns	ns	ns	ns	-			pos	pos	neg			pos	pos	neg		
730.24		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	neg	neg		
730.25		S	+	ns	ns	ns	ns	-			pos	rep	neg			pos	neg	neg		
730.6		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	neg			pos	inh/pos	ep(*pos		
730.8		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	pos			pos	neg	pos		
730.9		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	rep(*pos)			pos	rep	pos		
730.12		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.13		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	rep(*pos)			pos	pos	pos		
730.16		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	pos			pos	pos	pos		
730.23		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.26	7	S	+	S+ns	S+ns	S+ns	S+ns	+	5/5	5/5	inh/pos	inh	inh/pos	5/5	5/5	inh/pos	inh/neg	inh/pos	5/5	5/5
730.27		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	rep	rep(*pos)			pos	rep	pos		
730.28		S	+	S+ns	S+ns	S+ns	S+ns	+			inh/pos	inh	inh/pos			pos	pos	pos		
730.29		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		
730.30		S	+	S+ns	S+ns	S+ns	S+ns	+			pos	pos	pos			pos	pos	pos		

Data summary: number of positive samples/total number of samples (per level)

Annex N. POD-LOD calculations

(for information only)

Example of the spreadsheet results :

POD-LOD calculation program, version 7, dated 2016-03-01

This Excel program is distributed in the hope that it will be useful, but without any warranty. It can be downloaded at: www.wiwiss.fu-berlin.de/fachbereich/vwl/iso/ehemalige/wilrich/index.html where you can also subscribe to a newsletter informing about available updates.

How to use this program (Macros have to be enabled):

1. Enter the sample size, the total no. of matrices (up to 16) and the maximum no. of contamination levels for any of the matrices (up to 50) into the yellow cells. ► The appropriate no. of tables for your input data will be generated automatically below.

2. Enter your data into the yellow cells of the tables generated according to step 1.

3. Press 'Ctrl+b' to start the calculation. ► The results will be shown in green cells (also next to the input tables). Figures for the estimated probability of detection for each matrix and the combined results are shown in the sheets next to this sheet.

4. You can change the no. of matrices or levels in cell B23 or B24 at any time.

5. You can also change the data in the input tables and re-calculate with 'Ctrl+b'.

Examples corresponding to those in the publication are given in the sheets 'Example' and following. Further information on the underlying equations and the scope and application of this program can be found in the sheet 'Equations Info'.

Name of experiment:	Salmonella - PIF - Ref
Date of experiment:	
Sample size A_0 in g or ml:	100
Total no. of matrices:	1
Max. no. of contamination levels:	2

No. of matrix:	1	
Name of matrix/sample reference:	ref method	
Level of inoculum in cfu/g or cfu/ml	No. of inoculated tubes	No. of positive tubes
d	n	y
1,0	20	17
10,0	5	5

Results of the POD-LOD calculation

For details see: Wilrich & Wilrich, Journal of AOAC International, Vol. 92, No. 6, 2009, 1763-1772.

[1] $LOD_{50\%}$ = 50% Limit of detection, [2] $LOD_{95\%}$ = 95% Limit of detection

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1] Detection limit	$LOD_{50\%}$ [1] conf. limit	$LOD_{50\%}$ [1] conf. limit	$LOD_{95\%}$ [2] Detection limit	$LOD_{95\%}$ [2] conf. limit	$LOD_{95\%}$ [2] conf. limit	statistic matrix effect
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1	ref method	0,019	0,281	0,365	0,208	0,640	1,579	0,901	2,768	0,000
Combined data		0,019	0,281	0,365	0,208	0,640	1,579	0,901	2,768	0,000

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1] Detection limit	$LOD_{50\%}$ [1] Lower conf.	$LOD_{50\%}$ [1] Upper conf.	$LOD_{95\%}$ [2] Detection limit	$LOD_{95\%}$ [2] Lower conf.	$LOD_{95\%}$ [2] Upper conf.	statistic matrix effect
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1	ref method	0,019	0,281	0,365	0,208	0,640	1,579	0,901	2,768	0,000

Annex N. POD-LOD calculations, continued

Overview LOD for Reference method, and for the semi-automated DNA extraction using Magn Max Prep IV (PCR test kit A).

Salmonella	Ref method		
	$LOD_{50\%}$		
Item	Detection limit $D_{0.5,i}$	Lower conf. limit $D_{0.5,i,L}$	Upper conf. limit $D_{0.5,i,U}$
Infant formula	0,365	0,208	0,640
Infant formula containing probiotics	0,462	0,259	0,825
Starch	0,340	0,193	0,599
Vacuum cleaner residues	1,086	0,541	2,178

Salmonella	Magn Max Kit IV, PCR kit A		
	$LOD_{50\%}$		
Item	Detection limit $D_{0.5,i}$	Lower conf. limit $D_{0.5,i,L}$	Upper conf. limit $D_{0.5,i,U}$
Infant formula	0,365	0,208	0,640
Infant formula containing probiotics	0,462	0,259	0,825
Starch	0,340	0,193	0,599
Vacuum cleaner residues	1,269	0,623	2,584

NB Level of inoculum was based on inoculum counts made at the moment of inoculation (seeding), not at the moment of actual testing of the samples.

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Annex O. Tabulated raw data inclusivity testing

original data | newly generated data | additional data

Code	Strain		Ref. nr.	Origin	Source	Inoculum (cfu/sample)	Kit A result EB	Kit A result C	Kit A result Salm	Kit B result EB	Kit B result C	Kit B result Salm	Validation result
C1	*Salmonella*	Abaetetuba	Salm 712		Milk powder	5,5	pos	neg	pos	pos	neg	pos	as expected
S104	*Salmonella*	Adelaide	RIVM 7.14		Human	31	pos	neg	pos	pos	neg	pos	as expected
C3	*Salmonella*	Agona	Salm 698	Argentina	Feed: Sunflower scrap	6,2	pos	neg	pos	pos	neg	pos	as expected
C2	*Salmonella*	Agona (lactose pos)	Salm 686		Cream cake	9,3	pos	neg	pos	pos	neg	pos	as expected
C4	*Salmonella*	Alachua	Salm 709		Feed: Soybean scrap	7,0	pos	neg	pos	pos	neg	pos	as expected
S51	*Salmonella*	Albany	Salm 629	Venezuela	Foods	8	pos	neg	pos	pos	neg	pos	as expected
C5	*Salmonella*	Anatum	Salm 710	France	Milk powder	13,2	pos	neg	pos	pos	neg	pos	as expected
C6	*Salmonella*	Annedal	Salm 714	Ghana	Crushed melon seeds	3,9	pos	neg	pos	pos	neg	pos	as expected
S52	*Salmonella*	Arechavaleta	RIVM 19.8		Human	61	pos	neg	pos	pos	neg	pos	as expected
S53	*Salmonella*	Babelsberg	Salm 634	United Kingdom	Foods	48	pos	neg	pos	pos	neg	pos	as expected
S97	*Salmonella*	Baildon	RIVM SF20.1		Human	165	pos	neg	pos	pos	neg	pos	as expected
C9	*Salmonella*	Banana	Salm 717	Argentina	Feed: Soybean scrap	9,1	pos	neg	pos	pos	neg	pos	as expected
S54	*Salmonella*	Bangkok	Salm 636	Thailand	Foods	78	pos	neg	pos	pos	neg	pos	as expected
S55	*Salmonella*	Berta	RIVM 15.20		Chicken	60	pos	neg	pos	pos	neg	pos	as expected
S56	*Salmonella*	Blockley	RIVM 13-A.6		Meat products	42	pos	neg	pos	pos	neg	pos	as expected
S57	*Salmonella*	Bochum	RIVM 19.2		Environment	62	pos	neg	pos	pos	neg	pos	as expected
C10	*Salmonella*	Bovismorbificans	RIVM 7.15		Human	3,3	pos	neg	pos	pos	neg	pos	as expected
S59	*Salmonella*	Bracknell	RIVM 15.3		Chicken	37	pos	neg	pos	pos	neg	pos	as expected
S60	*Salmonella*	Braenderup	RIVM 16.16		Foods	59	pos	neg	pos	pos	neg	pos	as expected
S61	*Salmonella*	Brandenburg	Salm 638	Spain	Foods	46	pos	neg	pos	pos	neg	pos	as expected
C11	*Salmonella*	Bredeney	Salm 707		Environment	4,8	pos	neg	pos	pos	neg	pos	as expected
S62	*Salmonella*	Canstatt	RIVM 12.14		Animal feed	32	pos	neg	pos	pos	neg	pos	as expected
S63	*Salmonella*	Carno	RIVM 15.1		Pigs	40	pos	neg	pos	pos	neg	pos	as expected
C12	*Salmonella*	Cerro	Salm 680	Brazil	Feed: Soybean scrap	6,3	pos	neg	pos	pos	neg	pos	as expected
S64	*Salmonella*	Chester	RIVM 15.7		Human	53	pos	neg	pos	pos	neg	pos	as expected
C13	*Salmonella*	Corvallis	Salm 723		Environment	3,9	pos	neg	pos	pos	neg	pos	as expected
C14	*Salmonella*	Cubana	Salm 687		Cocoa (products)	3,8	pos	neg	pos	pos	neg	pos	as expected
C15	*Salmonella*	Derby	Salm 693	Germany	Spices	1,4	pos	neg	pos	pos	neg	pos	as expected
C16	*Salmonella*	Dublin	Salm 718		Cheese (raw milk)	3,8	pos	neg	pos	pos	neg	pos	as expected
C18	*Salmonella*	Durham	Salm 642	United Kingdom	Food	3,5	pos	neg	pos	pos	neg	pos	as expected
C19	*Salmonella*	Enteritidis	RIVM Stock	ATCC 13076*	Not stated	2,5	pos	neg	pos	pos	neg	pos	as expected
C20	*Salmonella*	Enteritidis PT 3	Salm 684		Eggs	4,4	pos	neg	pos	pos	neg	pos	as expected
S69	*Salmonella*	Frankfurt	Salm 644	Nigeria	Foods	23	pos	neg	pos	pos	neg	pos	as expected
S102	*Salmonella*	Goldcoast	RIVM 6.16		Human	15	pos	neg	pos	pos	neg	pos	as expected
C21	*Salmonella*	Hadar	Salm 645		Food	7,2	pos	neg	pos	pos	neg	pos	as expected
C22	*Salmonella*	Havana	Salm 678		Feed: Rapeseed scrap	8,2	pos	neg	pos	pos	neg	pos	as expected

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Annex O. Tabulated raw data inclusivity testing, continued

Code	Strain		Ref. nr.	Origin	Source	Inoculum (cfu/sample)	Kit A result EB	Kit A result C	Kit A result Salm	Kit B result EB	Kit B result C	Kit B result Salm	Validation result
S70	*Salmonella*	Heidelberg	RIVM 17.11		Bone meal	<1	pos	neg	pos	pos	neg	pos	as expected
C23	*Salmonella*	Indiana	Salm 721		Environment	1,7	pos	neg	pos	pos	neg	pos	as expected
C24	*Salmonella*	Infantis	Salm 682		Egg powder	11,2	pos	neg	pos	pos	neg	pos	as expected
C25	*Salmonella*	Infantis	Salm 716		Cheese (raw milk)	2,8	pos	neg	pos	pos	neg	pos	as expected
S71	*Salmonella*	Isangi	RIVM 17.2		Bone meal	45	pos	neg	pos	pos	neg	pos	as expected
S103	*Salmonella*	Jangwani	RIVM 7.2		Human	23	pos	neg	pos	pos	neg	pos	as expected
S107	*Salmonella*	Javiana	RIVM 21.P1		Human	19	pos	neg	pos	pos	neg	pos	as expected
S72	*Salmonella*	Kaapstad	Salm 651	Italy	Foods	40	pos	neg	pos	pos	neg	pos	as expected
S73	*Salmonella*	Kapemba	RIVM 14.14		Chicken	1	pos	neg	pos	pos	neg	pos	as expected
C26	*Salmonella*	Kentucky	Salm 696		Environment	13,9	pos	neg	pos	pos	neg	pos	as expected
S74	*Salmonella*	Kiambu	Salm 652	Ghana	Foods	68	pos	neg	pos	pos	neg	pos	as expected
S75	*Salmonella*	Kottbus	RIVM 17.10		Broilers	94	pos	neg	pos	pos	neg	pos	as expected
S76	*Salmonella*	Krefeld	RIVM 16.10		Animal feed	61	pos	neg	pos	pos	neg	pos	as expected
S77	*Salmonella*	Langenhorn	RIVM 19.4		Spices	56	pos	neg	pos	pos	neg	pos	as expected
S78	*Salmonella*	Langford	Salm 653	Spain	Foods	68	pos	neg	pos	pos	neg	pos	as expected
S79	*Salmonella*	Liverpool	Salm 654	Venezuela	Foods	36	pos	neg	pos	pos	neg	pos	as expected
C27	*Salmonella*	Livingstone	Salm 720		Dripping (fat)	5,4	pos	neg	pos	pos	neg	pos	as expected
S80	*Salmonella*	Llandoff	RIVM 14.1		Foods	69	pos	neg	pos	pos	neg	pos	as expected
C28	*Salmonella*	London	Salm 681		Environment	10,6	pos	neg	pos	pos	neg	pos	as expected
S81	*Salmonella*	Manchester	RIVM 12.3		Environmental swab	2	pos	neg	pos	pos	neg	pos	as expected
S98	*Salmonella*	Manhattan	MV3		Powdered product	73	pos	neg	pos	pos	neg	pos	as expected
C29	*Salmonella*	Mbandaka	Salm 695		Knuckles (for dogs)	9,9	pos	neg	pos	pos	neg	pos	as expected
S82	*Salmonella*	Miami	Salm 658	Guatamala	Foods	4	pos	neg	pos	pos	neg	pos	as expected
C30	*Salmonella*	Minnesota	Salm 697		Cattle Feed	10,7	pos	neg	pos	pos	neg	pos	as expected
C31	*Salmonella*	Mississippi	RIVM 14.15		Animal feed	6,2	pos	neg	pos	pos	neg	pos	as expected
C32	*Salmonella*	Montevideo	Salm 700		Environment	5,5	pos	neg	pos	pos	neg	pos	as expected
C34	*Salmonella*	Muenster	Salm 704		Artificial Calf Milk	2,5	pos	neg	pos	pos	neg	pos	as expected
C33	*Salmonella*	Muenster, H2S neg	Salm 386		Food	5,2	pos	neg	pos	pos	neg	pos	as expected
C35	*Salmonella*	Nagoya	Salm 689		Environment	3,6	pos	neg	pos	pos	neg	pos	as expected
S83	*Salmonella*	Napoli	Salm 660	Italy	Foods	96	pos	neg	pos	pos	neg	pos	as expected
C36	*Salmonella*	Newport	RIVM 12.7		Human	4,9	pos	neg	pos	pos	neg	pos	as expected
S105	*Salmonella*	Oakam	RIVM 8.11		Human	55	pos	neg	pos	pos	neg	pos	as expected
C37	*Salmonella*	Ohio	Salm 679		Poultry Feed	11,5	pos	neg	pos	pos	neg	pos	as expected
C38	*Salmonella*	Oranienburg	Salm 713	Ghana	Crushed melon seeds	5,5	pos	neg	pos	pos	neg	pos	as expected
S84	*Salmonella*	Orientalis	Salm 662	Singapore	Foods	7	pos	neg	pos	pos	neg	pos	as expected
S85	*Salmonella*	Orion	RIVM 17.18		Bone meal	78	pos	neg	pos	pos	neg	pos	as expected

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Annex O. Tabulated raw data inclusivity testing, continued

Code	Strain		Ref. nr.	Origin	Source	Inoculum (cfu/sample)	Kit A result EB	Kit A result C	Kit A result Salm	Kit B result EB	Kit B result C	Kit B result Salm	Validation result
C39	*Salmonella*	Panama	Salm 692		Milk powder	4,4	pos	neg	pos	pos	neg	pos	as expected
S86	*Salmonella*	Paratyphi A	Salm 774	LMG 16739	Not stated	22	pos	neg	pos	pos	neg	pos	as expected
S87	*Salmonella*	Paratyphi C	Salm 776	LMG 17134	Not stated	40	pos	neg	pos	pos	neg	pos	as expected
S88	*Salmonella*	Plymouth	RIVM 13.2		Others	61	pos	neg	pos	pos	neg	pos	as expected
S89	*Salmonella*	Poona	RIVM 17.5		Cornstarch	1	pos	neg	pos	pos	neg	pos	as expected
C40	*Salmonella*	Potsdam	Salm 715	Hungary	Grain	8,5	pos	neg	pos	pos	neg	pos	as expected
S90	*Salmonella*	Putten	RIVM 16.1		Bone meal	52	pos	neg	pos	pos	neg	pos	as expected
S91	*Salmonella*	Ramatgan	Salm 665	Singapore	Foods	135	pos	neg	pos	pos	neg	pos	as expected
S92	*Salmonella*	Richmond	Salm 666	Kenya	Foods	2	pos	neg	pos	pos	neg	pos	as expected
C41	*Salmonella*	Rissen	Salm 711		Feed: Sunflower scrap	5,6	pos	neg	pos	pos	neg	pos	as expected
S106	*Salmonella*	Ruiru	RIVM 8.20		Human	47	pos	neg	pos	pos	neg	pos	as expected
C42	*Salmonella*	Saintpaul	RIVM 8.10		Human	5,3	pos	neg	pos	pos	neg	pos	as expected
S93	*Salmonella*	Sandiego	Salm 668	Mexico	Foods	94	pos	neg	pos	pos	neg	pos	as expected
C43	*Salmonella*	Senftenberg	Salm 699	Spain	Spices	8,7	pos	neg	pos	pos	neg	pos	as expected
S94	*Salmonella*	Sherbrook	Salm 670	France	Foods	5	pos	neg	pos	pos	neg	pos	as expected
C44	*Salmonella*	Stanley	RIVM 14.5		Human	2,7	pos	neg	pos	pos	neg	pos	as expected
C45	*Salmonella*	Stockholm	RIVM 14.10		Cocoa beans	5,3	pos	neg	pos	pos	neg	pos	as expected
S95	*Salmonella*	Stourbridge	RIVM 16.5		Environment	105	pos	neg	pos	pos	neg	pos	as expected
S96	*Salmonella*	Taksony	RIVM 19.1		Others	64	pos	neg	pos	pos	neg	pos	as expected
C46	*Salmonella*	Tennessee	Salm 790		Human	7,5	pos	neg	pos	pos	neg	pos	as expected
C17	*Salmonella*	Thompson	RIVM 12.2		Animal feed	4,2	pos	neg	pos	pos	neg	pos	as expected
C47	*Salmonella*	Typhimurium	RIVM Stock	ATCC 14028*	Chicken liver/heart	3,6	pos	neg	pos	pos	neg	pos	as expected
C48	*Salmonella*	Typhimurium	Salm 722		Cheese	0,5	pos	neg	pos	pos	neg	pos	as expected
S99	*Salmonella*	Typhimurium, monophasic	RIVM 19.17	1,4,[5],12:i:-	Human	36	pos	neg	pos	pos	neg	pos	as expected
S101	*Salmonella*	Urbana	RIVM 19.6		Human	39	pos	neg	pos	pos	neg	pos	as expected
C49	*Salmonella*	Virchow	Salm 705	Ghana	Nuts	5,6	pos	neg	pos	pos	neg	pos	as expected
C50	*Salmonella*	Worthington	Salm 703		Environment	3,0	pos	neg	pos	pos	neg	pos	as expected
S100	*Salmonella*	Yaba	RIVM 19.6		Others	85	pos	neg	pos	pos	neg	pos	as expected
S65	*Salmonella*	*enterica subsp. arizonae* (IIIa)	Salm 633	Switzerland	Foods	11	pos	neg	pos	pos	neg	pos	as expected
C7	*Salmonella*	*enterica subsp. diarizonae* (IIIb)	Salm 631	Sri Lanka	Food	17,0	pos	neg	pos	pos	neg	pos	as expected
C8	*Salmonella*	*enterica subsp. diarizonae* (IIIb)	Salm 633	Switzerland	Food	1,2	pos	neg	pos	pos	neg	pos	as expected
S66	*Salmonella*	*enterica subsp. houtenae* (IV)	RIVM 17.21	44:z4,z32:-	Human	79	pos	neg	pos	pos	neg	pos	as expected
S67	*Salmonella*	*enterica subsp. indica* (VI)	MV 629	DSM 14848	Not stated	49	pos	neg	pos	pos	neg	pos	as expected
S68	*Salmonella*	*enterica subsp. salamae* (II)	RIVM 18.21	42:g,t:-	Kangaroo meat	3	pos	neg	pos	pos	neg	pos	as expected
S58	*Salmonella*	*bongori*	2011-2891	48:z35:-	Unknown	44	pos	neg	pos	pos	neg	pos	as expected

*As WDCM 00030 mentioned in ISO 6579 for quality assurance of culture media

** As WDCM 00031 mentioned in ISO 6579 for quality assurance of culture media

Annex P. Tabulated raw data exclusivity testing

(from the original study report)

									R 310 15	R 310 15	R 300 27	
					expected results in				LC 2.0	LC 2.0	LC 2.0	LC 2.0
Sample nr.	Strain	Ref. nr.	Origin	Source	EB	S	Inoculation per 90 ml BPW (cfu)	Date of DNA isolation	Part A-1 Result EB	Part A-1 Result (ES)	Part A-1 Result S	Validation Result Part A-1
C51	*Aeromonas hydrophila*	Micr 376	ATCC 7966	Milk	-	-	1,0E+07	21-10-2011	-	(-)	(-)	as expected
C52	*Bacillus cereus*	Micr 217		Infant formula	-	-	3,6E+05	24-10-2011	+/-	(-)	-/-	as expected
C65	*Enterococcus faecalis*	Micr 176	ATCC 29212*	Urine	-	-	1,5E+06	18-10-2011	-	(-)	(-)	as expected
C73	*Pseudomonas aeruginosa*	Micr 081	ATCC 27853*	Blood culture	-	-	1,7E+06	18-10-2011	-	(-)	(-)	as expected
C74	*Pseudomonas fluorescens*	Micr 377		Food	-	-	1,7E+06	21-10-2011	-	(-)	(-)	as expected
C75	*Pseudomonas putida*	Micr 379	ATCC 49128	Clinical isolate	-	-	1,2E+05	21-10-2011	-	(-)	(-)	as expected
C79	*Staphylococcus aureus*	Micr 381		Dairy: ice-cream	-	-	1,2E+06	21-10-2011	-	(-)	(-)	as expected
C62	*Enterobacter sakazakii*	Micr 372	Indonesia	Dairy: milk powder	+	-	5,9E+06	21-10-2011	+	(+)	-	as expected
C63	*Enterobacter sakazakii*	Micr 374	France	Infant formula	+	-	1,1E+07	21-10-2011	+	(+)	-	as expected
C64	*Enterobacter sakazakii*	Micr 489	Europe	Follow-up formula	+	-	1,2E+07	21-10-2011	+	(+)	-	as expected
C53	*Citrobacter braakii*	Micr 354		Dairy: cheese	+	-	4,5E+06	24-10-2011	+	(-)	-	as expected
C54	*Citrobacter freundii*	Micr 353		Food	+	-	4,1E+06	24-10-2011	+	(-)	-	as expected
C55	*Citrobacter koseri*	Micr 373	Korea	Dairy: milk powder	+	-	1,4E+07	21-10-2011	+	(-)	-	as expected
C56	*Citrobacter sedlakii*	Micr 371	UK	Food	+	-	1,4E+07	21-10-2011	+	(-)	-	as expected
C57	*Enterobacter amnigenus*	Micr 370	UK	Food	+	-	4,1E+06	24-10-2011	+	(-)	-	as expected
C58	*Enterobacter cloacae*	Micr 361		Infant formula	+	-	6,3E+06	18-10-2011	+	(-)	-	as expected
C59	*Enterobacter cloacae*	Micr 364	India	Environment: milk powder	+	-	4,8E+06	18-10-2011	+	(-)	-	as expected
C60	*Enterobacter helveticus*	Micr 363	Switzerland	Environment: milk powder	+	-	2,3E+06	18-10-2011	+	(-)	-	as expected
C61	*Enterobacter hormaechei*	Micr 375	India	Dairy: milk powder	+	-	2,1E+07	21-10-2011	+	(-)	-	as expected
C66	*Escherichia coli*	Micr 358		Dairy: cheese	+	-	4,1E+06	18-10-2011	+	(-)	-	as expected
C67	*Escherichia coli*	Micr 367	ATCC 25922*	Clinical isolate	+	-	7,4E+06	18-10-2011	+	(-)	-	as expected
C68	*Escherichia hermanii*	Micr 365	Germany	Environment: milk powder	+	-	2,8E+06	18-10-2011	+	(-)	-	as expected
C69	*Hafnia alvei*	Micr 356		Cosmetic: shampoo	+	-	1,2E+07	18-10-2011	+	(-)	-	as expected
C70	*Klebsiella oxytoca*	Micr 355		Dairy: cheese	+	-	2,0E+06	18-10-2011	+	(-)	-	as expected
C71	*Klebsiella pneumoniae*	Micr 359	Netherlands	Environment: milk powder	+	-	1,1E+06	18-10-2011	+	(-)	-	as expected
C72	*Proteus mirabilis*	Micr 357		Cosmetic: shampoo	+	-	4,3E+06	18-10-2011	+	(-)	-	as expected
C76	*Serratia ficaria*	Micr 362	Germany	Environment: milk powder	+	-	2,1E+06	18-10-2011	+	(-)	-	as expected
C77	*Serratia marcescens*	Micr 240	ATCC 274	Not stated	+	-	1,1E+07	21-10-2011	+	(-)	-	as expected
C78	*Shigella flexneri*	Micr 368	ATCC 12022	Not stated	+	-	2,8E+06	18-10-2011	+	(-)	-	as expected
C80	*Yersinia enterocolitica*	Micr 369	ATCC 9610	Human tissue	+	-	1,5E+06	18-10-2011	+	(-)	-	as expected

Annex Q. Raw data additional test kits and PCR machines

Q-1: part relative accuracy, relative specificity, relative sensitivity (from the original study report)

			R 310 15	R 300 27		R 310 15	R 310 27		R 310 15	R 310 27		R 302 15	R 302 27		R 302 15	R 302 27		R 302 15	R 302 27	
			LC 2.0	LC 2.0		LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5
Sample nr.	Type	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result S	Validation Result Part A-1	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B
121	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	-	NA	-	(-)	NA
123	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	-	NA			
124	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
125	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
129	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	rep				
130	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	rep				
131	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
132	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	rep				
133	CPa	-	-	(-)	NA	-	(rep)	NA	+	-	NA	-	(-)	NA	rep	-	NA			
136	CPa	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
179	CPb	-	rep/-	(+)	NA	rep/-	(rep)	NA	-	(-)	NA	rep	-	NA	-	(-)	NA			
183	CPb	-	-	(+)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
184	CPb	-	-	(+)	NA	-	(rep)	NA	-	(-)	NA	rep	-	NA	rep	-	NA			
187	CPb	-	-	(+)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
191	CPb	-	rep/-	(+)	NA	rep/-	(rep)	NA	-	(-)	NA	rep	-	NA	rep	-	NA	-	(-)	NA
192	CPb	-	-	(+)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
165	CPc	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
166	CPc	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
169	CPc	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
170	CPc	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
173	CPc	-	-	(+)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
172	CPc	-	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
176	CPc	-	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
155	CPd	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	rep		-	(-)	NA
156	CPd	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
164	CPd	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
195	CPd	-	-	(-)	NA															
196	CPd	-	-	(-)	NA															
197	CPd	-	-	(-)	NA															
4	FF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	-	(-)	NA			

Annex Q. Raw data additional test kits and PCR machines, continued

Q-1: part relative accuracy, relative specificity, relative sensitivity (from the original study report)

			R 310 15	R 300 27		R 310 15	R 310 27		R 310 15	R 310 27		R 302 15	R 302 27		R 302 15	R 302 27		R 302 15	R 302 27	
			LC 2.0	LC 2.0		LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5
Sample nr.	Type	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result S	Validation Result Part A-1	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B
5	FF	-	-	(-)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA			
33	FF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
38	FF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
43	FF	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
2	FF	-	-	(rep)	NA	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA			
9	FF	-	-	(rep)	NA	-	(rep)	NA	-	(-)	NA	-	(rep)	NA	-	(rep)	NA			
32	FFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
95	FFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	-	NA			
99	FFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
117	FFpP	-	-	(-)	NA	-	(-)	NA	+	-	NA	-	(-)	NA	rep	-	NA			
119	FFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	-	NA			
198	FFpP	-	-	(-)	NA															
199	FFpP	-	-	(-)	NA															
1	FFpP	-	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-		NA			
31	FFpP	-	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	+	rep		+	rep				
34	IF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
35	IF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
37	IF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
44	IF	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
3	IF	-	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	+	rep		+	rep				
36	IF	-	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
39	IFpP	-	-	(-)	NA	-	(-)	NA	-	(rep)	NA	-	(-)	NA		(-)	NA	-	(-)	NA
40	IFpP	-	-	(-)	NA	-	(rep)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA			
46	IFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
91	IFpP	-	-	(-)	NA	-	(rep)	NA	-	(rep)	NA	-	(-)	NA	rep	-	NA			
106	IFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	-	NA			
107	IFpP	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(rep)	NA	rep	-	NA	-	(-)	NA
115	IFpP	-	-	(-)	NA	-	(rep)	NA	-	(rep)	NA	-	(-)	NA	rep	-	NA	-	(-)	NA
103	IFpP	-	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	rep	-	NA			
11	Starch	-	+	-	NA	+	rep		+	rep		+	rep		+	-	NA			

Annex Q. Raw data additional test kits and PCR machines, continued

Q-1: part relative accuracy, relative specificity, relative sensitivity (from the original study report)

			R 310 15	R 300 27		R 310 15	R 310 27		R 310 15	R 310 27		R 302 15	R 302 27		R 302 15	R 302 27		R 302 15	R 302 27	
			LC 2.0	LC 2.0		LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5
Sample nr.	Type	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result S	Validation Result Part A-1	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B
12	Starch	-	+	-	NA	+	-	NA	+	-	NA	+	rep		+	-	NA			
13	Starch	-	+	-	NA	+	rep		+	rep		+	rep		+	-	NA			
30	Starch	-	+	-	NA	+	-	NA	+	-	NA	+	-	NA	+	-	NA			
24	Starch	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
25	Starch	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
26	Starch	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
29	Starch	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
14	Starch	-	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(-)	NA			
15	Starch	-	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA	-	(rep)	NA			
72	Sucrose	-	+	-	NA	+	-	NA	+	-	NA	+	-	NA	+	-	NA			
73	Sucrose	-	+	-	NA	+	-	NA	+	-	NA	+	-	NA	+	-	NA			
74	Sucrose	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
75	Sucrose	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
76	Sucrose	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
77	Sucrose	-	-	(-)	NA	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA			
78	Sucrose	-	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
79	Sucrose	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
86	Sucrose	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
80	Sucrose	-	-	(rep)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
54	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
56	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA			
57	Vitamins	-	-	(-)	NA	-	(rep)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA			
61	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
64	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
65	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
69	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
70	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA	-	(-)	NA
138	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(rep)	NA	-	(-)	NA	-	(-)	NA			
139	Vitamins	-	-	(-)	NA	-	(-)	NA	-	(-)	NA	rep	rep		rep	-	NA	-	(-)	NA
126	CPa	+/-	-/-	(-)/(-)	ND	-/-	(-)	ND	-	(-)	ND	-	(-)	ND	rep	-	ND	-	(-)	ND

Annex Q. Raw data additional test kits and PCR machines, continued

Q-1: part relative accuracy, relative specificity, relative sensitivity (from the original study report)

			R 310 15	R 300 27		R 310 15	R 310 27		R 310 15	R 310 27		R 302 15	R 302 27		R 302 15	R 302 27		R 302 15	R 302 27	
			LC 2.0	LC 2.0		LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5
Sample nr.	Type	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result S	Validation Result Part A-1	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B
48	FF	+/+	-/-	(-)/(-)	ND	-/-	(-)	ND	-	(-)	ND	-	(-)	ND	-	(-)	ND	-	(-)	ND
8	IF	+/+	-/-	(rep)/(-)	ND	-/-	(rep)	ND	+	rep		+/+	rep		+/+	rep		+	+	PA
18	Starch	+/+	rep/-	(rep)/(-)	ND	rep/-	+	PA	+	rep		+	+	PA	+	+	PA	+	+	PA
122	CPa	+	+	+	PA	+	+	PA	+	+	PA	+	empty		empty	empty				
127	CPa	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
128	CPa	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
134	CPa	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
135	CPa	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
180	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
181	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
182	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
186	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
188	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
189	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
190	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
193	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
194	CPb	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
167	CPc	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
168	CPc	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
171	CPc	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
174	CPc	+	+	+	PA	+	rep		+	+	PA	+	+	PA	+	+	PA			
175	CPc	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
177	CPc	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
178	CPc	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
151	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
157	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
158	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
159	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
160	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
161	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			

Annex Q. Raw data additional test kits and PCR machines, continued

Q-1: part relative accuracy, relative specificity, relative sensitivity (from the original study report)

			R 310 15	R 300 27		R 310 15	R 310 27		R 310 15	R 310 27		R 302 15	R 302 27		R 302 15	R 302 27		R 302 15	R 302 27	
			LC 2.0	LC 2.0		LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5
Sample nr.	Type	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result S	Validation Result Part A-1	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B
162	CPd	+	+	+	PA	+	rep		+	+	PA	+	+	PA	+	+	PA			
163	CPd	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
10	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
41	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
42	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
141	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
143	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
146	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	rep	+	PA			
148	FF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
98	FFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
100	FFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
102	FFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
113	FFpP	+	+	+	PA	+	inhibition		-	(inhibition)	ND	+	+	PA	+	+	PA			
114	FFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
118	FFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
49	IF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
50	IF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
142	IF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
144	IF	+	+	+	PA	+	+	PA	+	+	PA	+	empty		empty	empty				
145	IF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
147	IF	+	+	+	PA	+	+	PA	+	+	PA	+	empty		empty	+	PA			
149	IF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
150	IF	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
92	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
93	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
94	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
104	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
108	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
110	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
112	IFpP	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			

Annex Q. Raw data additional test kits and PCR machines, continued

Q-1: part relative accuracy, relative specificity, relative sensitivity (from the original study report)

			R 310 15	R 300 27		R 310 15	R 310 27		R 310 15	R 310 27		R 302 15	R 302 27		R 302 15	R 302 27		R 302 15	R 302 27	
			LC 2.0	LC 2.0		LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5
Sample nr.	Type	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result S	Validation Result Part A-1	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part A-2 Result EB	Part A-2 Result S	Validation results Part A-2	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B	Part B Result EB	Part B Result S	Validation results Part B
16	Starch	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
17	Starch	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
19	Starch	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
20	Starch	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
27	Starch	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
28	Starch	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
200	Starch	+	+	+	PA															
201	Starch	+	+	+	PA															
202	Starch	+	+	+	PA															
81	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
82	Sucrose	+	+	+	PA	+	inhibition		inhibition	rep		+	+	PA	+	+	PA			
83	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
84	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
85	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
87	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
88	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
89	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
90	Sucrose	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
208	Sucrose	+	+	+	PA															
52	Vitamins	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
58	Vitamins	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
59	Vitamins	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA			
62	Vitamins	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
140	Vitamins	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA	+	+	PA
203	Vitamins	+	+	+	PA															
204	Vitamins	+	+	+	PA															
205	Vitamins	+	+	+	PA															
211	Vitamins	+	+	+	PA															
212	Vitamins	+	+	+	PA															
185	CPb	-	+	+	PD	+	+	PD	+	+	PD	+	+	PD	+	+	PD			
				PA	86		PA	74		PA	75		PA	75		PA	76		PA	14
				NA	89		NA	82		NA	82		NA	78		NA	78		NA	21
				PD	1		PD	1		PD	1		PD	1		PD	1		PD	0
				ND	4		ND	3		ND	3		ND	2		ND	2		ND	2
	Total	180		Total	180		Total	160		Total	161		Total	156		Total	157		Total	37

Annex Q. Raw data additional test kits and PCR machines, continued

Q-2: part relative detection level study (from the original study report)

		R 310 15	R 310 15	R 300 27	R 310 15	R 310 15	R 310 27	R 310 15	R 310 15	R 310 27	R 302 15	R 302 15	R 302 27	R 302 15	R 302 15	R 302 27
		LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p
Sample nr.	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result (ES)	Part A-1 Result S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Part B Result EB	Part B Result (ES)	Part B Result S	Part B Result EB	Part B Result (ES)	Part B Result S
B 4	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 6	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 7	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 12	-	+	(+)	rep/-	+	(+)	rep	+	(+)	rep	+	(+)	rep	+	(+)	rep
B 13	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 15	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 20	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 25	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 26	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 30	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-	+	(+)	-
B 1	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 2	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 3	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 5	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 8	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 9	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 10	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 11	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)
B 16	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 17	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 18	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 19	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)
B 22	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 28	-	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 61	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 62	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 63	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 64	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 65	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 66	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 67	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 72	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 75	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)
B 80	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 91	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)

Annex Q. Raw data additional test kits and PCR machines, continued

Q-2: part relative detection level study (from the original study report)

		R 310 15	R 310 15	R 300 27	R 310 15	R 310 15	R 310 27	R 310 15	R 310 15	R 310 27	R 302 15	R 302 15	R 302 27	R 302 15	R 302 15	R 302 27
		LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p
Sample nr.	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result (ES)	Part A-1 Result S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Part B Result EB	Part B Result (ES)	Part B Result S	Part B Result EB	Part B Result (ES)	Part B Result S
B 92	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 93	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 94	-	-	(-)	(rep)/(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 95	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 96	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 97	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 98	-	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 99	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 100	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 102	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 103	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 105	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 106	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 107	-	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 109	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 112	-	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 113	-	-	(-)	(-)	-	(-)	(rep)	-	(-)	(-)	-	(-)	(-)	-	(-)	(-)
B 14	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 21	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 23	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 24	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 27	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 29	+	+	(-)	+	+	(-)	+	+	(+)	+	+	(+)	+	+	(+)	+
B 31	+	+	(+)	+	+	(+)	+	+	(+)	+	+	(+)	+	+	(+)	+
B 32	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 33	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 34	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 35	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 36	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 37	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 38	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 39	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 40	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 41	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 42	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+

Annex Q. Raw data additional test kits and PCR machines, continued

Q-2: part relative detection level study (from the original study report)

		R 310 15	R 310 15	R 300 27	R 310 15	R 310 15	R 310 27	R 310 15	R 310 15	R 310 27	R 302 15	R 302 15	R 302 27	R 302 15	R 302 15	R 302 27
		LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p
Sample nr.	REF Final confirmed result	Part A-1 Result EB	Part A-1 Result (ES)	Part A-1 Result S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Part B Result EB	Part B Result (ES)	Part B Result S	Part B Result EB	Part B Result (ES)	Part B Result S
B 68	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 69	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 70	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 71	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 73	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 74	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 76	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 77	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 78	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 79	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 81	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 82	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 83	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 84	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 85	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 86	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 87	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 88	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 89	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 90	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 101	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 104	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 108	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 110	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 111	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 114	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 115	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 116	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 117	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 118	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 119	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+
B 120	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+	+	(-)	+

Annex Q. Raw data additional test kits and PCR machines, continued

Q-3: part inclusivity study (from the original study report)

	Expected results		R 310 15	R 310 15	R 310 27		R 310 15	R 310 15	R 310 27		R 302 15	R 302 15	R 302 27		R 302 15	R 302 15	R 302 27		R 302 15	R 302 15	R 302 27	
			LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5	iQ5
Strain tested	EB	S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Validation Result Part A-2	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Validation Result Part A-2	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B
Abaetetuba	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Agona (lactose pos)	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Agona	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Alachua	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Anatum	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Annedal	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Arizonae (Group IIIb)	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Arizonae (Group IIIb)	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Banana	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Bovismorbificans	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Bredeney	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Cerro	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Corvallis	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Cubana	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Derby	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Dublin	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Duisburg	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Durham	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Enteritidis	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Enteritidis PT 3	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Hadar	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Havana	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Indiana	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Infantis	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Infantis	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Kentucky	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Livingstone	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
London	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Mbandaka	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Minnesota	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected

Annex Q. Raw data additional test kits and PCR machines, continued

Q-3: part inclusivity study (from the original study report)

	Expected results		R 310 15	R 310 15	R 310 27		R 310 15	R 310 15	R 310 27		R 302 15	R 302 15	R 302 27		R 302 15	R 302 15	R 302 27		R 302 15	R 302 15	R 302 27	
			LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5	iQ5
Strain tested	EB	S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Validation Result Part A-2	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Validation Result Part A-2	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B
Mississippi	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Montevideo	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Muenster, H2S neg	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Muenster	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Nagoya	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Newport	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Ohio	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Oranienburg	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Panama	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Potsdam	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Rissen	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Saintpaul	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Senftenberg	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Stanley	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Stockholm	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Tennessee	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Typhimurium	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Typhimurium	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Virchow	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected
Worthington	pos	pos	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected	+	(-)	+	as expected

Annex Q. Raw data additional test kits and PCR machines, continued

Q-4: part exclusivity study (from the original study report)

	Expected results		R 310 15	R 310 15	R 310 27		R 310 15	R 310 15	R 310 27		R 302 15	R 302 15	R 302 27		R 302 15	R 302 15	R 302 27		R 302 15	R 302 15	R 302 27	
			LC 2.0	LC 2.0	LC 2.0	LC 2.0	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	LC 480	mx3005p	mx3005p	mx3005p	mx3005p	iQ5	iQ5	iQ5	iQ5
Strain tested	EB	S	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Validation Result Part A-2	Part A-2 Result EB	part A-2 Result (ES)	Part A-2 Result S	Validation Result Part A-2	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B	Part B Result EB	Part B Result (ES)	Part B Result S	Validation Result Part B
Aeromonas hydrophila	neg	neg	-	(-)	(inhibition	as expected	-	(-)	(inhibition	as expected	-	(-)	(rep)	as expected	rep	(-)	rep		-	(-)	(-)	as expected
Bacillus cereus	neg	neg	+/-	(-)	-	as expected	+	(-)	-	as expected	+	(-)	rep		+	(-)	-	as expected	+	(-)	-	as expected
Enterococcus faecalis	neg	neg	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(rep)	as expected	-	(-)	(rep)	as expected	-	(-)	(-)	as expected
Pseudomonas aeruginosa	neg	neg	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected
Pseudomonas fluorescens	neg	neg	-	(-)	(rep)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected
Pseudomonas putida	neg	neg	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected
Staphylococcus aureus	neg	neg	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected	-	(-)	(-)	as expected
Enterobacter sakazakii	pos	neg	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected
Enterobacter sakazakii	pos	neg	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected
Enterobacter sakazakii	pos	neg	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected	+	(+)	-	as expected
Citrobacter braakii	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	rep		+	(-)	rep		+	(-)	-	as expected
Citrobacter freundii	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Citrobacter koseri	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	rep		+	(-)	rep		+	(-)	-	as expected
Citrobacter sedlakii	pos	neg	+	(-)	rep		+	(-)	rep		+	(-)	rep		+	(-)	rep		+	(-)	rep	
Enterobacter amnigenus	pos	neg	+	(-)	rep		+	(-)	-	as expected	+	(-)	rep		+	(-)	-	as expected	+	(-)	-	as expected
Enterobacter cloacae	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Enterobacter cloacae	pos	neg	+	(-)	rep		+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Enterobacter helveticus	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Enterobacter hormaechei	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Escherichia coli	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	rep		+	(-)	-	as expected
Escherichia coli	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Escherichia hermanii	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Hafnia alvei	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Klebsiella oxytoca	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Klebsiella pneumoniae	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	rep		+	(-)	-	as expected	+	(-)	-	as expected
Proteus mirabilis	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Serratia ficaria	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Serratia marcescens	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	rep		+	(-)	-	as expected	+	(-)	-	as expected
Shigella flexneri	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected
Yersinia enterocolitica	pos	neg	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected	+	(-)	-	as expected

Annex R. Overview ILS results all participants and EL

(from the original study report)

Sample code	Inoculation level	Expected result	Results lab B			Results lab D			Results lab H		
			REF	ALT LC 2.0		REF	ALT LC 2.0		REF	ALT LC 2.0	
				EB (R310 15)	Salm (R 300 27)		EB (R310 15)	Salm (R 300 27)		EB (R310 15)	Salm (R 300 27)
EBESS1	L0	neg	-	-	(-)	-	-	(rep/-)	-	-	(rep/+)
EBESS4	L0	neg	-	-	(-)	-	-	(rep/-)	-	-	-
EBESS9	L0	neg	-	-	(-)	-	-	(rep/-)	-	+	rep/+
EBESS12	L0	neg	-	-	(-)	-	-	(rep/-)	-	-	(rep/+)
EBESS14	L0	neg	-	-	(-)	-	-	(rep/-)	-	-	(-)
EBESS17	L0	neg	-	-	(-)	-	-	(-)	+/-	-	(-)
EBESS18	L0	neg	-	-	(-)	-	-	(-)	-	-	(rep/+)
EBESS24	L0	neg	-	-	(-)	-	-	(-)	+/+	-	(+)
EBESS3	L1	pos	+	+	+	+	+	+	+	+	+
EBESS5	L1	pos	+	+	+	+	+	+	+	+	+
EBESS8	L1	pos	+	+	+	+	+	+	+	+	+
EBESS13	L1	pos	+	+	+	+	+	+	+	+	+
EBESS15	L1	pos	-	-	(-)	+	+	+	+	+	+
EBESS19	L1	pos	+	+	+	+	+	+	+	+	+
EBESS21	L1	pos	+	+	+	+	+	+	+	+	+
EBESS23	L1	pos	+	+	+	+	+	+	+	+	+
EBESS2	L2	pos	+	+	+	+	+	+	+	+	+
EBESS6	L2	pos	+	+	+	+	+	+	+	+	+
EBESS7	L2	pos	+	+	+	+	+	+	+	+	+
EBESS10	L2	pos	+	+	+	+	+	+	+	+	+
EBESS11	L2	pos	+	+	+	+	+	+	+	+	+
EBESS16	L2	pos	+	+	+	+	+	+	+	+	+
EBESS20	L2	pos	+	+	+	+	+	+	+	+	+
EBESS22	L2	pos	+	+	+	+	+	+	+	+	+

Sample code	Inoculation level	Expected result	Results lab C			Results lab O		
			REF	ALT LC 2.0/480		REF	ALT LC 480	
				EB (R310 15)	Salm (R 310 27)		EB (R310 15)	Salm (R 310 27)
EBESS1	L0	neg	-	-	(-)	-	-	(-)
EBESS4	L0	neg	-	-	(-)	-	-	(-)
EBESS9	L0	neg	-	-	(-)	-	-	(-)
EBESS12	L0	neg	-	-	(-)	-	-	(-)
EBESS14	L0	neg	-	-	(-)	-	-	(-)
EBESS17	L0	neg	-	-	(-)	-	-	(-)
EBESS18	L0	neg	-	-	(-)	-	-	(-)
EBESS24	L0	neg	-	-	(-)	-	-	(-)
EBESS3	L1	pos	+	+	+	+	+	+
EBESS5	L1	pos	+	+	+	+	+	+
EBESS8	L1	pos	+	+	+	+	+	+
EBESS13	L1	pos	+	+	+	+	+	+
EBESS15	L1	pos	-	-	(-)	+	+	+
EBESS19	L1	pos	+	+	+	+	+	+
EBESS21	L1	pos	+	+	+	+	+	+
EBESS23	L1	pos	+	+	+	+	+	+
EBESS2	L2	pos	+	+	+	+	+	+
EBESS6	L2	pos	+	+	+	+	+	+
EBESS7	L2	pos	+	+	+	+	+	+
EBESS10	L2	pos	+	+	+	+	+	+
EBESS11	L2	pos	+	+	+	+	+	+
EBESS16	L2	pos	+	+	+	+	+	+
EBESS20	L2	pos	+	+	+	+	+	+
EBESS22	L2	pos	+	+	+	+	+	+

Sample code	Inoculation level	Expected result	Results Expert Lab		
			REF	ALT LC 2.0	
				EB (R310 15)	Salm (R 300 27)
EBESS1	L0	neg	-	-	(-)
EBESS4	L0	neg	-	-	(-)
EBESS9	L0	neg	-	-	(-)
EBESS12	L0	neg	-	-	(-)
EBESS14	L0	neg	-	-	(-)
EBESS17	L0	neg	-	-	(-)
EBESS18	L0	neg	-	-	(-)
EBESS24	L0	neg	-	-	(-)
EBESS3	L1	pos	-	-	(-)
EBESS5	L1	pos	+	+	+
EBESS8	L1	pos	+	+	+
EBESS13	L1	pos	+	+	+
EBESS15	L1	pos	-	-	(-)
EBESS19	L1	pos	+	+	+
EBESS21	L1	pos	+	+	+
EBESS23	L1	pos	+	+	+
EBESS2	L2	pos	+	+	+
EBESS6	L2	pos	+	+	+
EBESS7	L2	pos	+	+	+
EBESS10	L2	pos	+	+	+
EBESS11	L2	pos	+	+	+
EBESS16	L2	pos	+	+	+
EBESS20	L2	pos	+	+	+
EBESS22	L2	pos	+	+	+

			Results lab A			Results lab E			Results lab F			Results lab G			Results lab I			Results lab J			Results lab K			Results lab L			Results lab M			Results lab N		
				ALT			ALT			ALT			ALT			ALT			ALT			ALT			ALT			ALT			ALT		
				Chromo 4			Mx3005p			Biorad CFX			Biorad CFX4			Rotorgene Q			Mx3005p			Mx3005p			LC 480			Chromo 4			iQ5		
Sample code	Inoculation level	Expected result	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)	REF	EB (R302 15)	Salm (R 302 27)
EBESS1	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)
EBESS4	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)
EBESS9	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	+/+	+/+	+/+	-	-	(-)	-	-	(-)
EBESS12	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	+/+	-	(-)	-	-	(-)	-	-	(-)	+/-	-/-	(-)/-	-	-	(-)	-	-	(-)
EBESS14	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	+/-	-/-	(-)/-	-	-	(-)	-	-	(-)
EBESS17	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	+/+	-/+	(-)/+	-	-	(-)	-	-	(-)
EBESS18	L0	neg	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-/-	+/-	+/-	-	-	(-)
EBESS24	L0	neg	-	-	(-)	-	+	-	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)	-	-	(-)
EBESS3	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	-	-	(-)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS5	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	(-)	+	+	+	+	+	+
EBESS8	L1	pos	-	-	(-)	-	-	(-)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS13	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS15	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS19	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS21	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	(-)	+	+	+	+	+	+
EBESS23	L1	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	(-)	+	+	+	+	+	+
EBESS2	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS6	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS7	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS10	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS11	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS16	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS20	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EBESS22	L2	pos	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(-)	Results for Salmonella PCR between brackets, this would not have been tested during standard routine testing because the EB PCR was already negative
-	Negative result for an inoculated sample, but consistent result for reference method and alternative method
(rep/+)	Unexpected positive result for Salmonella PCR, but would not have been tested during standard routine testing (EB PCR-negative)
+	Presumptive positive result by alternative method but non-confirmed by culture
+/+	Positive result reference method for a non-inoculated sample, inconsistent with the alternative method result
+/+	Positive result reference method for a non-inoculated sample, but consistent with the alternative method result