Summary Report for MicroVal certificate 2009 LR8/9/19/20, concerning the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 15.1 and R 302 15.1) for detection of *Enterobacteriaceae* and/ or *Cronobacter* spp. in Infant formula and infant cereals, Probiotics containing products, Ingredients and Environmental samples

MicroVal study number: 2009LR 8/9/19/20
Alternative methods: the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 15.1 and R 302 15.1)

Summary Report: final version 28-01-2019

Contact Expert Laboratory:
W. Jacobs-Reitsma
National Institute for Public Health and the Environment (RIVM)
Centre for Zoonoses and Environmental Microbiology (Z&O; Pb 63)
PO box 1
3720 BA Bilthoven
The Netherlands
e-mail: wilma.jacobs@rivm.nl

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

Authors: W. Jacobs-Reitsma, R. Diddens, E. Bouw, A. Gritchina, W. van Overbeek
Company: BIOTECON Diagnostics GmbH, Potsdam, Germany.
Expert Laboratory: RIVM-Z&O (National Institute for Public Health and the Environment, Centre for Zoonoses and Environmental Microbiology), Bilthoven, The Netherlands.

Kit names:
foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 15.1 and R 302 15.1)

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:
ISO 21528-1:2017 Microbiology of the food chain - Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection of *Enterobacteriaceae*

ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* 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
CSB	*Cronobacter* Screening Broth
CCI	Chromogenic *Cronobacter* Isolation agar
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 *Enterobacteriaceae* and *Cronobacter*
EL	Expert Laboratory
FP	False Positive
FPR	False Positive Ratio
g	gram
h	hour
ILS	Interlaboratory Study
inh	inhibition
LOD	Level of Detection
MCS	Method Comparison Study
min	minute
ml	milliliter
MVTC	MicroVal Technical Committee
NA	Negative Agreement
na	not applicable
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)
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
VRBG	Violet Red Bile Glucose agar

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

	Enterobacteriaceae	*Enterobacteriaceae*
	Cronobacter	*Cronobacter*
	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

Contents

1 Introduction

In this project the **renewal** of MicroVal certificate 2009LR8/9/19/20 (detection of *Enterobacteriaceae* and/or *Cronobacter* spp., based on RIKILT confidential report R2010.506*)* was carried out by RIVM-Z&O as the MicroVal Expert Laboratory.

Note that the original certificate/report still used the name "*E. sakazakii*" which nowadays is referred to as "*Cronobacter*".

In addition, this renewal also included the following *extensions*:
-New Category: Ingredients, using the original DNA extraction method (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 DNA extraction step (StarPrep One, BIOTECON Diagnostics Cat. No. S 400 07).

The separately reported extension to this certificate (May 2018, MicroVal report TC 2018-063) 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).
These data are incorporated in this renewal report as well, to give a complete overview of the current situation of certificate 2009 LR 8/9/19/20.

The alternative methods for combined detection of *Enterobacteriaceae* and *Cronobacter* spp. are:

Alternative method **A**:

- 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:

- Reagent D (BIOTECON Diagnostics Cat. No. A 500 02) plus the **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07), being the original DNA extraction method.

- Reagent D (BIOTECON Diagnostics Cat. No. A 500 02) plus the **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15), being the extended (semi-automated) DNA extraction method.

Alternative method **B**:

- 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:

- Reagent D (BIOTECON Diagnostics Cat. No. A 500 02) plus the **food**proof® StarPrep One Kit (BIOTECON Diagnostics Cat. No. S 400 07), being the original DNA extraction method.

- Reagent D (BIOTECON Diagnostics Cat. No. A 500 02) plus the **food**proof® Magnetic Preparation Kit IV (BIOTECON Diagnostics Cat. No. S 400 15), being the extended (semi-automated) DNA extraction method.

The reference methods are:
EN-ISO 21528-1:2017 Microbiology of the food chain - Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection of *Enterobacteriaceae*

EN-ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

Previous versions of the reference methods (ISO 21528-1:2004, ISO/TS 22964:2006) were used in the original LR 8/9/19/20 validation study in 2009. Revised versions of these ISO standards were published in 2017. The main changes introduced in both these documents compared to the previous versions are considered as **major**. Therefore, the Sensitivity Study and the Relative level of detection Study were repeated, using the latest versions of the reference methods, to replace those data in the original validation study.

Scope of the validation study (Categories):

Certificate 2009LR8/9/19/20	2018 Renewal - Extensions
Powdered infant formula	Infant formula and infant cereals
Infant formula containing probiotics and/or cereals	Probiotics containing products
	Ingredients
Environmental samples	Environmental samples

Note that the scope of the renewal study, as supported by the newly generated data for the Sensitivity Study and the Relative level of detection Study, was slightly modified to align with closely related certificate 2011LR39.

Criteria evaluated during the study have been:

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

This report corresponds to the method comparison study and the interlaboratory study, and gathers the observed data and interpretations according to the EN ISO 16140- 2:2016 standard and the most recent version of the MicroVal technical committee interpretations.

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

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® StarPrep One Kit for manual DNA extraction, shows comparable performance to the reference method EN-ISO 21528-1:2017 for the detection of *Enterobacteriaceae* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® StarPrep One Kit for manual DNA extraction, shows comparable performance to the reference method EN-ISO 22964:2017 for the detection of *Cronobacter* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® Magnetic Preparation Kit IV for semi-automated DNA extraction, shows comparable performance to the reference method EN-ISO 21528-1:2017 for the detection of *Enterobacteriaceae* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® Magnetic Preparation Kit IV for

semi-automated DNA extraction, shows comparable performance to the reference method EN-ISO 22964:2017 for the detection of *Cronobacter* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The overview on the claims for the current LR8/9/19/20 certificate is given in the Table below:

Detection of *Enterobacteriacea* and/or detection of *Cronobacter* spp.

	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	**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			
	Thermocyclers	LC 480 II	LC 2.0	LC 480 II	IQ5	ABI 7500	Mx 3005	LC 480 II	LC 2.0	LC 480 II	IQ5	ABI 7500	Mx 3005
	Software version	1.5.1	4.1	1.5.1	2.0	2.0.6	MX4.1d	1.5.1	4.1	1.5.1	2.0	2.0.6	MX4.1d
Scope (including subcultivation Protocol)	Infant formula and infant cereals (P1)	X	X	X	X	X	X	X	X	X	X	X	X
	Probiotics containing products (P1)	X	X	X	X	X	X	X	X	X	X	X	X
	Probiotics containing products (P2)	X	X	X	X	X	X	X	X	X	X	X	X
	Ingredients (P1)	X	X	X	X	X	X	X	X	X	X	X	X
	Environmental samples (P1)	X	X	X	X	X	X	X	X	X	X	X	X

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

2 Method Protocols

2.1 Reference methods

See the flow diagram 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 semi-automated DNA extraction.
Presence of *Enterobacteriaceae* and presence of *Cronobacter* spp. is tested concurrently 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).
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).

2.3 Study design

The Method Comparison Study was 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.
However, both the revised ISO 21528:2017 on detection of *Enterobacteriaceae* and the revised ISO 22964:2017 on detection of *Cronobacter* were validated for 10 gram test portions only and it was questioned if these revised reference methods would also work for 100 gram test portions. The RLOD approach was used to test both 10 gram and 100 gram samples of the most challenging type (probiotics-containing infant formula). All data (also see Annex J) were sent to the MVTC for discussion at their 30th meeting. The RLOD for *Cronobacter* detection was found to be 0,559 and for *Enterobacteriaceae* detection 0,626. This data was accepted by the MVTC to proof that 100 gram samples can be used for the reference method as well.
Another question on the reference methods 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

methods are 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. 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.

After discussing the questions above, the MVTC decided (30th meeting) that the study could be conducted as a **paired** study. 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-H. In case of a “repetition” result, guidelines as now given in the updated kit inserts were followed.

For the recent extension/renewal 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)(kit A, hybridization probes) and on an iQ5 (Bio-Rad) (kit B, 5’Nuclease). 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 ABI 7500 (Applied Biosystems) for test kit B, based on testing blindly-coded DNA samples by an external laboratory (BIOTECON Diagnostics, Potsdam, Germany).

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 method and the alternative method 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.

A total of 272 samples were analyzed for both *Enterobacteriaceae* and *Cronobacter*. Upon request of the report reviewers and after discussion in the MVTC (35th meeting) an additional set of 19 samples were tested for presence of *Enterobacteriaceae*. These samples were artificially inoculated with 10 different strains of *Enterobacteriaceae*, not being *Cronobacter* or *Salmonella*, to extend on the wider variety of *Enterobacteriaceae* strains under study (also see Annex K).

Table 1 - Categories, types and number of samples analyzed, 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 for *EB*	Number of samples for *Cronobacter*
				1	2			
1	Infant formula and infant cereals	a	Infant formula (intended for infants < 1 year)	x		100 g	26	22
		b	Infant formula (intended for infants > 1 year)	x		100 g	26	21
		c	Infant cereals	x		100 g	21	21
2	Probiotic containing products	a	Probiotic infant formula	x	x	100 g	34	24
		b	Probiotic infant cereals	x	x	100 g	22	22
		c	Probiotic ingredients	x	x	100 g	20	20
3	Ingredients	a	Standard ingredients	x		100 g	22	22
		b	Infant cereals ingredients	x		100 g	26	26
		c	Premixes	x		12,5 g	28	28
4	Environmental samples	a	Sweep samples/equipment swabs	x		swabs	24	24
		b	Traject samples (in-line factory)	x		100 g	22	22
		c	Vacuum cleaner residues	x		100 g	20	20

[1] **Alternative method protocols:**

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

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 kit A and PCR test kit B.

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

Category		Type	Positive* samples *EB*	Negative samples *EB*	Total *EB*	Positive* samples *Cronobacter*	Negative samples *Cronobacter*	Total *Cronobacter*
1	Infant formula and infant cereals	a	16	10	26	10	12	22
		b	15	11	26	10	11	21
		c	15	6	21	12	9	21
		Total	46	27	73	32	32	64
2.1/ 2.2	Probiotic containing products	a	21	13	34	11	13	24
		b	12	10	22	10	12	22
		c	11	9	20	9	11	20
		Total	44	32	76	30	36	66
3	Ingredients	a	13	9	22	10	12	22
		b	20	6	26	13	13	26
		c	15	13	28	11	17	28
		Total	48	28	76	34	42	76
4	Environmental samples	a	16	8	24	11	13	24
		b	13	9	22	11	11	22
		c	13	7	20	11	9	20
		Total	42	24	66	33	33	66
Total			180	111	291	129	143	272

*Positive by at least one of the methods

3.1.2 Test sample preparation

17 samples (9,4%) were found to be naturally contaminated with *Enterobacteriaceae*, (also see Annex L). 5 samples (3,9%) were found to be naturally contaminated with *Cronobacter*.

Naturally contaminated samples were preferentially analyzed, but due to the nature of the samples it was clear that artificial contaminations were needed as well.
Details on the artificial contaminations are presented in Annex K. In most cases a combination of a *Cronobacter* and a *Salmonella* strain was used, indicated by “combi number” in Annex L with the raw data and further details are given in Annex K.
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 *Cronobacter* lenticule (drying/freezing stress) was used for direct inoculation (spiking).

172 samples were artificially contaminated with *Enterobacteriaceae*, using 31 different *Cronobacter* strains and 38 different *Salmonella* strains, plus an additional 10 different *Enterobacteriaceae* strains. 163 of those gave a positive result for *Enterobacteriaceae*.

147 samples were artificially contaminated with *Cronobacter*, using 31 different *Cronobacter* strains. 124 of those gave a positive result for *Cronobacter*.

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

3.1.3 Confirmation protocols

As the validation study was considered to be a paired study, the confirmation of the alternative method was using the confirmation results of the reference method, as performed on the initial BPW enrichment.
Confirmation of *Enterobacteriaceae* was done according to ISO 21528-1 (Oxidase, Glucose).
Confirmation of *Cronobacter* was done according to ISO 22964 (biochemical confirmation by using the ID 32E identification test kit).

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 the ISO method as appropriate. The first result and a result after re-testing (if applicable) are both indicated in Annex L (for example ns/S or -/+).

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, StarPrep One Kit and the MagPrep IV, for all Categories (1, 2.1 or 2.2, 3 and 4), and PCR test kit A and test kit B, are given in Table 3-S/3-M and Table 4-S/4-M for *Enterobacteriaceae*, and in Table 5-S/5-M and Table 6-S/6-M for *Cronobacter*.

Table 3-S - Summary of sensitivity study results: StarPrep One Kit, all categories (1, 2.1, 3, 4) – *Enterobacteriaceae*

Test kit:		Reference method positive (R+)	Reference method negative (R-)
Test kit A	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 177	Positive deviation (R-/A+) PD = 0
Test kit A	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 3	Negative agreement (R-/A-) NA = 111
Test kit B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 176	Positive deviation (R-/A+) PD = 0
Test kit B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 4	Negative agreement (R-/A-) NA = 111

Table 4-S - Summary of sensitivity study results: StarPrep One Kit, all categories (1, 2.2, 3, 4) – *Enterobacteriaceae* (identical results PCR test kit A and PCR test kit B)

Test Kit:		Reference method positive (R+)	Reference method negative (R-)
A/B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 176	Positive deviation (R-/A+) PD = 0
A/B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 4	Negative agreement (R-/A-) NA = 111

Table 3-M/Table 4-M - Summary of sensitivity study results: MagPrep IV, all categories (1, 2.1/2.2, 3, 4) – *Enterobacteriaceae* (Identical result for PCR Test kit A and test kit B)

Test Kit:		Reference method positive (R+)	Reference method negative (R-)
A/B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 176	Positive deviation (R-/A+) PD = 0
A/B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 4	Negative agreement (R-/A-) NA = 111

Table 5-S - Summary of sensitivity study results: StarPrep One Kit, all categories (1, 2.1, 3, 4) – *Cronobacter* (identical results for PCR kit A and PCR kit B)

Test Kit:		Reference method positive (R+)	Reference method negative (R-)
A/B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 126	Positive deviation (R-/A+) PD = 1
A/B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 2	Negative agreement (R-/A-) NA = 143

Table 6-S - Summary of sensitivity study results: StarPrep One Kit, all categories (1, 2.2, 3, 4) – *Cronobacter*

Test kit:		Reference method positive (R+)	Reference method negative (R-)
Test kit A	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 123	Positive deviation (R-/A+) PD = 1
Test kit A	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 5	Negative agreement (R-/A-) NA = 143
Test kit B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 124	Positive deviation (R-/A+) PD = 1
Test kit B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 4	Negative agreement (R-/A-) NA = 143

Table 5-M - Summary of sensitivity study results: MagPrep IV, all categories (1, 2.1, 3, 4) – *Cronobacter*

Test kit:		Reference method positive (R+)	Reference method negative (R-)
Test kit A	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 124	Positive deviation (R-/A+) PD = 1
Test kit A	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 4	Negative agreement (R-/A-) NA = 143
Test kit B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 125	Positive deviation (R-/A+) PD = 1
Test kit B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 3	Negative agreement (R-/A-) NA = 143

Table 6-M - Summary of sensitivity study results: MagPrep IV, all categories (1, 2.2, 3, 4) – *Cronobacter*

Test kit:		Reference method positive (R+)	Reference method negative (R-)
Test kit A	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 122	Positive deviation (R-/A+) PD = 1
Test kit A	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 6	Negative agreement (R-/A-) NA = 143
Test kit B	Alternative method positive (A+)	Positive agreement (R+/A+) PA = 123	Positive deviation (R-/A+) PD = 1
Test kit B	Alternative method negative (A-)	Negative deviation (R+/A-) ND = 5	Negative agreement (R-/A-) NA = 143

Tables 7 (PCR test kit A) and Tables 8 (PCR test kit B) show the interpretation of sample results between the reference and alternative methods (based on the confirmed alternative methods), for each of the alternative method protocols under study for *Enterobacteriaceae*.
Tables 9 (PCR test kit A) and Tables 10 (PCR test kit B) show the interpretation of sample results between the reference and alternative methods (based on the confirmed alternative methods), for each of the alternative method protocols under study for *Cronobacter*.

Table 7-S - Interpretation of sample results: StarPrep One Kit, PCR test kit A, *Enterobacteriaceae*

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)	16	10	0	0	0	0	26
		b	Infant formula (intended for infants > 1 year)	14	11	0	1	0	0	26
		c	Infant cereals	15	6	0	0	0	0	21
		Total		45	27	0	1	0	0	73
2.1	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	0	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	0	0	20
		Total		44	32	0	0	0	0	76
2.2	Probiotic containing products	a	Probiotic infant formula	20	13	0	1	0	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	1	0	20
		Total		43	32	0	1	1	0	76
3	Ingredients	a	Standard ingredients	12	9	0	1	0	0	22
		b	Infant cereals ingredients	19	6	0	1	0	0	26
		c	Premixes	15	13	0	0	0	0	28
		Total		46	28	0	2	0	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	16	8	0	0	1	0	24
		b	Traject samples (in-line factory)	13	9	0	0	0	0	22
		c	Vacuum cleaner residues	13	7	0	0	0	0	20
		Total		42	24	0	0	1	0	66
ALL Categories (1, 2.1, 3, 4)				177	111	0	3	1	0	291
ALL Categories (1, 2.2, 3, 4)				176	111	0	4	2	0	291

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

Table 8-S - Interpretation of sample results: StarPrep One Kit, PCR test kit B, *Enterobacteriaceae*

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)	15	10	0	1	0	0	26
		b	Infant formula (intended for infants > 1 year)	14	11	0	1	0	0	26
		c	Infant cereals	15	6	0	0	0	0	21
		Total		44	27	0	2	0	0	73
2.1	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	0	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	0	0	20
		Total		44	32	0	0	0	0	76
2.2	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	0	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	1	0	20
		Total		44	32	0	0	1	0	76
3	Ingredients	a	Standard ingredients	12	9	0	1	0	0	22
		b	Infant cereals ingredients	19	6	0	1	0	0	26
		c	Premixes	15	13	0	0	0	0	28
		Total		46	28	0	2	0	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	16	8	0	0	1	0	24
		b	Traject samples (in-line factory)	13	9	0	0	0	0	22
		c	Vacuum cleaner residues	13	7	0	0	1	0	20
		Total		42	24	0	0	2	0	66
ALL Categories (1, 2.1, 3, 4)				176	111	0	4	2	0	291
ALL Categories (1, 2.2, 3, 4)				176	111	0	4	3	0	291

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

Table 7-M - Interpretation of sample results: Mag Prep IV, PCR test kit A, *Enterobacteriaceae*

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)	16	10	0	0	0	0	26
		b	Infant formula (intended for infants > 1 year)	14	11	0	1	0	0	26
		c	Infant cereals	15	6	0	0	1	0	21
		Total		45	27	0	1	1	0	73
2.1	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	0	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	0	0	20
		Total		44	32	0	0	0	0	76
2.2	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	1	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	1	0	20
		Total		44	32	0	0	2	0	76
3	Ingredients	a	Standard ingredients	12	9	0	1	0	0	22
		b	Infant cereals ingredients	19	6	0	1	0	0	26
		c	Premixes	14	13	0	1	0	0	28
		Total		45	28	0	3	0	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	16	8	0	0	1	0	24
		b	Traject samples (in-line factory)	13	9	0	0	0	0	22
		c	Vacuum cleaner residues	13	7	0	0	0	0	20
		Total		42	24	0	0	1	0	66
ALL Categories (1, 2.1, 3, 4)				176	111	0	4	2	0	291
ALL Categories (1, 2.2, 3, 4)				176	111	0	4	4	0	291

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

Table 8-M - Interpretation of sample results: MagPrep IV, PCR test kit B, *Enterobacteriaceae*

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)	16	10	0	0	0	0	26
		b	Infant formula (intended for infants > 1 year)	14	11	0	1	0	0	26
		c	Infant cereals	15	6	0	0	0	0	21
		Total		45	27	0	1	0	0	73
2.1	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	0	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	0	0	20
		Total		44	32	0	0	0	0	76
2.2	Probiotic containing products	a	Probiotic infant formula	21	13	0	0	1	0	34
		b	Probiotic infant cereals	12	10	0	0	0	0	22
		c	Probiotic ingredients	11	9	0	0	1	0	20
		Total		44	32	0	0	2	0	76
3	Ingredients	a	Standard ingredients	12	9	0	1	0	0	22
		b	Infant cereals ingredients	19	6	0	1	0	0	26
		c	Premixes	14	13	0	1	0	0	28
		Total		45	28	0	3	0	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	16	8	0	0	1	0	24
		b	Traject samples (in-line factory)	13	9	0	0	0	0	22
		c	Vacuum cleaner residues	13	7	0	0	0	0	20
		Total		42	24	0	0	1	0	66
ALL Categories (1, 2.1, 3, 4)				176	111	0	4	1	0	291
ALL Categories (1, 2.2, 3, 4)				176	111	0	4	3	0	291

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

Table 9-S - Interpretation of sample results: StarPrep One Kit, PCR test kit A, *Cronobacter*

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	12	0	1	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	11	0	1	0	0	21
		c	Infant cereals	12	9	0	0	0	0	21
		Total		30	32	0	2	0	0	64
2.1	Probiotic containing products	a	Probiotic infant formula	11	13	0	0	0	0	24
		b	Probiotic infant cereals	10	12	0	0	0	0	22
		c	Probiotic ingredients	8	11	1	0	1	0	20
		Total		29	36	1	0	1	0	66
2.2	Probiotic containing products	a	Probiotic infant formula	9	13	0	2	0	0	24
		b	Probiotic infant cereals	10	12	0	0	1	0	22
		c	Probiotic ingredients	7	11	1	1	1	0	20
		Total		26	36	1	3	2	0	66
3	Ingredients	a	Standard ingredients	10	12	0	0	0	0	22
		b	Infant cereals ingredients	13	13	0	0	2	0	26
		c	Premixes	11	17	0	0	0	0	28
		Total		34	42	0	0	2	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	11	13	0	0	0	0	24
		b	Traject samples (in-line factory)	11	11	0	0	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		33	33	0	0	0	0	66
ALL Categories (1, 2.1, 3, 4)				126	143	1	2	3	0	272
ALL Categories (1, 2.2, 3, 4)				123	143	1	5	4	0	272

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

Table 10-S - Interpretation of sample results: StarPrep One Kit, PCR test kit B, *Cronobacter*

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	12	0	0	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	11	0	1	0	0	21
		c	Infant cereals	11	9	0	1	0	0	21
		Total		30	32	0	2	0	0	64
2.1	Probiotic containing products	a	Probiotic infant formula	11	13	0	0	0	0	24
		b	Probiotic infant cereals	10	12	0	0	0	0	22
		c	Probiotic ingredients	8	11	1	0	1	0	20
		Total		29	36	1	0	1	0	66
2.2	Probiotic containing products	a	Probiotic infant formula	10	13	0	1	0	0	24
		b	Probiotic infant cereals	10	12	0	0	0	0	22
		c	Probiotic ingredients	7	11	1	1	1	0	20
		Total		27	36	1	2	1	0	66
3	Ingredients	a	Standard ingredients	10	12	0	0	0	0	22
		b	Infant cereals ingredients	13	13	0	0	2	0	26
		c	Premixes	11	17	0	0	0	0	28
		Total		34	42	0	0	2	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	11	13	0	0	0	0	24
		b	Traject samples (in-line factory)	11	11	0	0	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		33	33	0	0	0	0	66
ALL Categories (1, 2.1, 3, 4)				126	143	1	2	3	0	272
ALL Categories (1, 2.2, 3, 4)				124	143	1	4	3	0	272

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

Table 9-M - Interpretation of sample results: MagPrep IV, PCR test kit A, *Cronobacter*

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	12	0	1	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	11	0	1	0	0	21
		c	Infant cereals	12	9	0	0	0	0	21
		Total		30	32	0	2	0	0	64
2.1	Probiotic containing products	a	Probiotic infant formula	11	13	0	0	0	0	24
		b	Probiotic infant cereals	10	12	0	0	0	0	22
		c	Probiotic ingredients	8	11	1	0	0	0	20
		Total		29	36	1	0	0	0	66
2.2	Probiotic containing products	a	Probiotic infant formula	11	13	0	0	0	0	24
		b	Probiotic infant cereals	8	12	0	2	0	0	22
		c	Probiotic ingredients	8	11	1	0	2	0	20
		Total		27	36	1	2	2	0	66
3	Ingredients	a	Standard ingredients	10	12	0	0	0	0	22
		b	Infant cereals ingredients	13	13	0	0	1	0	26
		c	Premixes	10	17	0	1	0	0	28
		Total		33	42	0	1	1	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	11	13	0	0	0	0	24
		b	Traject samples (in-line factory)	10	11	0	1	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		32	33	0	1	0	0	66
ALL Categories (1, 2.1, 3, 4)				124	143	1	4	1	0	272
ALL Categories (1, 2.2, 3, 4)				122	143	1	6	3	0	272

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

Table 10-M - Interpretation of sample results: MagPrep IV, PCR test kit B, *Cronobacter*

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	12	0	1	0	0	22
		b	Infant formula (intended for infants > 1 year)	9	11	0	1	0	0	21
		c	Infant cereals	12	9	0	0	1	0	21
		Total		30	32	0	2	1	0	64
2.1	Probiotic containing products	a	Probiotic infant formula	11	13	0	0	0	0	24
		b	Probiotic infant cereals	10	12	0	0	0	0	22
		c	Probiotic ingredients	8	11	1	0	0	0	20
		Total		29	36	1	0	0	0	66
2.2	Probiotic containing products	a	Probiotic infant formula	11	13	0	0	0	0	24
		b	Probiotic infant cereals	8	12	0	2	0	0	22
		c	Probiotic ingredients	8	11	1	0	2	0	20
		Total		27	36	1	2	2	0	66
3	Ingredients	a	Standard ingredients	10	12	0	0	0	0	22
		b	Infant cereals ingredients	13	13	0	0	2	0	26
		c	Premixes	11	17	0	0	0	0	28
		Total		34	42	0	0	2	0	76
4	Environmental samples	a	Sweep samples/equipment swabs	11	13	0	0	0	0	24
		b	Traject samples (in-line factory)	10	11	0	1	0	0	22
		c	Vacuum cleaner residues	11	9	0	0	0	0	20
		Total		32	33	0	1	0	0	66
ALL Categories (1, 2.1, 3, 4)				125	143	1	3	3	0	272
ALL Categories (1, 2.2, 3, 4)				123	143	1	5	5	0	272

[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 11 were calculated for all Categories and Types, and for each of the alternative method protocols under study.

Tables 12 (PCR test kit A) and Tables 13 (PCR test kit B) give the overview for *Enterobacteriaceae*.
Tables 14 (PCR test kit A) and Tables 15 (PCR test kit B) give the overview for *Cronobacter*.

Table 11 – 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 12-S – Interpretation of sample results: StarPrep One Kit, PCR test kit A, *Enterobacteriaceae*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	16	10	0	0	0	100%	100,0%	100,0%	0,0%
	b	14	11	0	1	0	93,3%	100,0%	96,2%	0,0%
	c	15	6	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	45	27	0	1	0	97,8%	100,0%	98,6%	0,0%
2.1	a	21	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	12	10	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	44	32	0	0	0	100,0%	100,0%	100,0%	0,0%
2.2	a	20	13	0	1	0	95,2%	100,0%	97,1%	0,0%
	b	12	10	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	43	32	0	1	1	97,7%	100,0%	98,7%	3,1%
3	a	12	9	0	1	0	92,3%	100,0%	95,5%	0,0%
	b	19	6	0	1	0	95,0%	100,0%	96,2%	0,0%
	c	15	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	46	28	0	2	0	95,8%	100,0%	97,4%	0,0%
4	a	16	8	0	0	1	100,0%	100,0%	100,0%	12,5%
	b	13	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	13	7	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	42	24	0	0	1	100,0%	100,0%	100,0%	4,2%
ALL Cats (with 2.1)		177	111	0	3	1	98,3%	100,0%	99,0%	0,9%
ALL Cats (with 2.2)		176	111	0	4	2	97,8%	100,0%	98,6%	1,8%

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

Table 13-S - Interpretation of sample results: StarPrep One Kit, PCR test kit B, *Enterobacteriaceae*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	15	10	0	1	0	93,8%	100,0%	96,2%	0,0%
	b	14	11	0	1	0	93,3%	100,0%	96,2%	0,0%
	c	15	6	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	44	27	0	2	0	95,7%	100,0%	97,3%	0,0%
2.1	a	21	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	12	10	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	44	32	0	0	0	100,0%	100,0%	100,0%	0,0%
2.2	a	21	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	12	10	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	44	32	0	0	1	100,0%	100,0%	100,0%	3,1%
3	a	12	9	0	1	0	92,3%	100,0%	95,5%	0,0%
	b	19	6	0	1	0	95,0%	100,0%	96,2%	0,0%
	c	15	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	46	28	0	2	0	95,8%	100,0%	97,4%	0,0%
4	a	16	8	0	0	1	100,0%	100,0%	100,0%	12,5%
	b	13	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	13	7	0	0	1	100,0%	100,0%	100,0%	14,3%
	Total	42	24	0	0	2	100,0%	100,0%	100,0%	8,3%
ALL Cats (with 2.1)		176	111	0	4	2	97,8%	100,0%	98,6%	1,8%
ALL Cats (with 2.2)		176	111	0	4	3	97,8%	100,0%	98,6%	2,7%

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

Table 12-M - Interpretation of sample results: Mag Prep IV, PCR test kit A, *Enterobacteriaceae*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	16	10	0	0	0	100%	100,0%	100,0%	0,0%
	b	14	11	0	1	0	93,3%	100,0%	96,2%	0,0%
	c	15	6	0	0	1	100,0%	100,0%	100,0%	16,7%
	Total	45	27	0	1	1	97,8%	100,0%	98,6%	3,7%
2.1	a	21	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	12	10	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	44	32	0	0	0	100,0%	100,0%	100,0%	0,0%
2.2	a	21	13	0	0	1	100,0%	100,0%	100,0%	7,7%
	b	12	10	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	44	32	0	0	2	100,0%	100,0%	100,0%	6,3%
3	a	12	9	0	1	0	92,3%	100,0%	95,5%	0,0%
	b	19	6	0	1	0	95,0%	100,0%	96,2%	0,0%
	c	14	13	0	1	0	93,3%	100,0%	96,4%	0,0%
	Total	45	28	0	3	0	93,8%	100,0%	96,1%	0,0%
4	a	16	8	0	0	1	100,0%	100,0%	100,0%	12,5%
	b	13	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	13	7	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	42	24	0	0	1	100,0%	100,0%	100,0%	4,2%
ALL Cats (with 2.1)		176	111	0	4	2	97,8%	100,0%	98,6%	1,8%
ALL Cats (with 2.2)		176	111	0	4	4	97,8%	100,0%	98,6%	3,6%

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

Table 13-M - Interpretation of sample results: MagPrep IV, PCR test kit B, *Enterobacteriaceae*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	16	10	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	14	11	0	1	0	93,3%	100,0%	96,2%	0,0%
	c	15	6	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	45	27	0	1	0	97,8%	100,0%	98,6%	0,0%
2.1	a	21	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	12	10	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	44	32	0	0	0	100,0%	100,0%	100,0%	0,0%
2.2	a	21	13	0	0	1	100,0%	100,0%	100,0%	7,7%
	b	12	10	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	44	32	0	0	2	100,0%	100,0%	100,0%	6,3%
3	a	12	9	0	1	0	92,3%	100,0%	95,5%	0,0%
	b	19	6	0	1	0	95,0%	100,0%	96,2%	0,0%
	c	14	13	0	1	0	93,3%	100,0%	96,4%	0,0%
	Total	45	28	0	3	0	93,8%	100,0%	96,1%	0,0%
4	a	16	8	0	0	1	100,0%	100,0%	100,0%	12,5%
	b	13	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	13	7	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	42	24	0	0	1	100,0%	100,0%	100,0%	4,2%
ALL Cats (with 2.1)		176	111	0	4	1	97,8%	100,0%	98,6%	0,9%
ALL Cats (with 2.2)		176	111	0	4	3	97,8%	100,0%	98,6%	2,7%

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

Table 14-S - Interpretation of sample results: StarPrep One Kit, PCR test kit A, *Cronobacter*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	9	12	0	1	0	90,0%	100,0%	95,5%	0,0%
	b	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	c	12	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	30	32	0	2	0	93,8%	100,0%	96,9%	0,0%
2.1	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	8	11	1	0	1	100,0%	88,9%	95,0%	9,1%
	Total	29	36	1	0	1	100,0%	96,7%	98,5%	2,8%
2.2	a	9	13	0	2	0	81,8%	100,0%	91,7%	0,0%
	b	10	12	0	0	1	100,0%	100,0%	100,0%	8,3%
	c	7	11	1	1	1	88,9%	88,9%	90,0%	9,1%
	Total	26	36	1	3	2	90,0%	96,7%	93,9%	5,6%
3	a	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	13	13	0	0	2	100,0%	100,0%	100,0%	15,4%
	c	11	17	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	34	42	0	0	2	100,0%	100,0%	100,0%	4,8%
4	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	11	11	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	33	33	0	0	0	100,0%	100,0%	100,0%	0,0%
ALL Cats (with 2.1)		126	143	1	2	3	98,4%	99,2%	98,9%	2,1%
ALL Cats (with 2.2)		123	143	1	5	4	96,1%	99,2%	97,8%	2,8%

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

Table 15-S - Interpretation of sample results: StarPrep One Kit, PCR test kit B, *Cronobacter*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	c	11	9	0	1	0	91,7%	100,0%	95,2%	0,0%
	Total	30	32	0	2	0	93,8%	100,0%	96,9%	0,0%
2.1	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	8	11	1	0	1	100,0%	88,9%	95,0%	9,1%
	Total	29	36	1	0	1	100,0%	96,7%	98,5%	2,8%
2.2	a	10	13	0	1	0	90,9%	100,0%	95,8%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	7	11	1	1	1	88,9%	88,9%	90,0%	9,1%
	Total	27	36	1	2	1	93,3%	96,7%	95,5%	2,8%
3	a	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	13	13	0	0	2	100,0%	100,0%	100,0%	15,4%
	c	11	17	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	34	42	0	0	2	100,0%	100,0%	100,0%	4,8%
4	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	11	11	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	33	33	0	0	0	100,0%	100,0%	100,0%	0,0%
ALL Cats (with 2.1)		126	143	1	2	3	98,4%	99,2%	98,9%	2,1%
ALL Cats (with 2.2)		124	143	1	4	3	96,9%	99,2%	98,2%	2,1%

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

Table 14-M - Interpretation of sample results: MagPrep IV, PCR test kit A, *Cronobacter*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	9	12	0	1	0	90,0%	100,0%	95,5%	0,0%
	b	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	c	12	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	30	32	0	2	0	93,8%	100,0%	96,9%	0,0%
2.1	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	8	11	1	0	0	100,0%	88,9%	95,0%	0,0%
	Total	29	36	1	0	0	100,0%	96,7%	98,5%	0,0%
2.2	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	8	12	0	2	0	80,0%	100,0%	90,9%	0,0%
	c	8	11	1	0	2	100,0%	88,9%	95,0%	18,2%
	Total	27	36	1	2	2	93,3%	96,7%	95,5%	5,6%
3	a	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	13	13	0	0	1	100,0%	100,0%	100,0%	7,7%
	c	10	17	0	1	0	90,9%	100,0%	96,4%	0,0%
	Total	33	42	0	1	1	97,1%	100,0%	98,7%	2,4%
4	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	10	11	0	1	0	90,9%	100,0%	95,5%	0,0%
	c	11	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	32	33	0	1	0	97,0%	100,0%	98,5%	0,0%
ALL Cats (with 2.1)		124	143	1	4	1	96,9%	99,2%	98,2%	0,7%
ALL Cats (with 2.2)		122	143	1	6	3	95,3%	99,2%	97,4%	2,1%

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

Table 15-M - Interpretation of sample results: MagPrep IV PCR test kit B, *Cronobacter*

Category	Type	PA	NA[1]	PD	ND[2]	FP[3]	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
1	a	9	12	0	1	0	90,0%	100,0%	95,5%	0,0%
	b	9	11	0	1	0	90,0%	100,0%	95,2%	0,0%
	c	12	9	0	0	1	100,0%	100,0%	100,0%	11,1%
	Total	30	32	0	2	1	93,8%	100,0%	96,9%	3,1%
2.1	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	c	8	11	1	0	0	100,0%	88,9%	95,0%	0,0%
	Total	29	36	1	0	0	100,0%	96,7%	98,5%	0,0%
2.2	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	8	12	0	2	0	80,0%	100,0%	90,9%	0,0%
	c	8	11	1	0	2	100,0%	88,9%	95,0%	18,2%
	Total	27	36	1	2	2	93,3%	96,7%	95,5%	5,6%
3	a	10	12	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	13	13	0	0	2	100,0%	100,0%	100,0%	15,4%
	c	11	17	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	34	42	0	0	2	100,0%	100,0%	100,0%	4,8%
4	a	11	13	0	0	0	100,0%	100,0%	100,0%	0,0%
	b	10	11	0	1	0	90,9%	100,0%	95,5%	0,0%
	c	11	9	0	0	0	100,0%	100,0%	100,0%	0,0%
	Total	32	33	0	1	0	97,0%	100,0%	98,5%	0,0%
ALL Cats (with 2.1)		125	143	1	3	3	97,7%	99,2%	98,5%	2,1%
ALL Cats (with 2.2)		123	143	1	5	5	96,1%	99,2%	97,8%	3,5%

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

3.1.6 Discordant results

Negative deviations for *Enterobacteriacea*, for each of the alternative method protocols under study, are listed in Table 16.

Negative deviations for *Cronobacter*, for each of the alternative method protocols under study, are listed in Table 17.

Table 16 - Negative deviations: *Enterobacteriaceae*

			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	Inoculation (CFU/Sample)
1a	51	1	(PA)	ND	(PA)	(PA)	-	+	Crono: 2,5 & Salm: 0,7
1b	214	1	ND	ND	ND	ND	-	+	Crono: <1 & Salm: < 1
3a	88	1	ND	ND	ND	ND	-	+	na
3b	191	1	ND	ND	ND	ND	-	+	na
3c	279	1	(PA)	(PA)	ND	(PA)	-	+	Crono: 6
2b	158	2	ND	(PA)	(PA)	(PA)	-	+	Crono: 2,4 & Salm: 1,6

Table 17 - Negative deviations: *Cronobacter*

			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	Inoculation (CFU/Sample)
1c	50	1	(PA)	ND	(PA)	(PA)	-	+	Crono: <1 & Salm: 1,7
1a	242	1	ND	(PA)	ND	ND	-	+	Crono: 4,9 & Salm: < 1
1b	214	1	ND	ND	ND	ND	-	+	Crono: <1 & Salm: < 1
3c	279	1	(PA)	(PA)	ND	(PA)	-	+	Crono: 6
4b	271	1	(PA)	(PA)	ND	ND	-	+	Crono: 1,7 & Salm: 1
2a	153	2	ND	ND	(PA)	(PA)	-	+	Crono: 2,4 & Salm: 1,6
2a	158	2	ND	(PA)	(PA)	(PA)	-	+	Crono: 2,4 & Salm: 1,6
2b	252	2	(PA)	(PA)	ND	ND	-	+	Crono: 4,6 & Salm: 2,8
2b	253	2	(PA)	(PA)	ND	ND	-	+	Crono: <1 & Salm: 4
2c	171	2	ND	ND	(PA)	(PA)	-	+	Crono: <1 & Salm: < 1

No Positive deviations for *Enterobacteriacea* were found.

Positive deviations for *Cronobacter*, for each of the alternative method protocols under study, are listed in Table 18, all concerning the same sample (n°173).

Table 18 - Positive deviations: *Cronobacter*

			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	Inoculation (CFU/Sample)
2c	173	1	PD	PD	PD	PD	+	+*	Crono: 1,6 & Salm: 3,3
2c	173	2	PD	PD	PD	PD	+	+*	Crono: 1,6 & Salm: 3,3

* by re-testing 10 ml cold-stored initial BPW using ISO 22964

The analysis of discordant results according to ISO 16140-2:2016 for a **paired** study is given in Tables 19 – Tables 22.

Table 19-S - Interpretation of sensitivity study results: StarPrep One Kit, PCR test kit A, *Enterobacteriaceae*

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

[1] ND: including PPND

Table 20-S - Interpretation of sensitivity study results: StarPrep One Kit, PCR test kit B, *Enterobacteriaceae*

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	0	0	0	3	0	6
2.2	0	0	0	3	0	6
3	2	0	2	3	2	6
4	0	0	0	3	0	6
All Categories (1, 2.1, 3, 4)	4	0	4	5	4	12
All Categories (1, 2.2, 3, 4)	4	0	4	5	4	12

[1] ND: including PPND

Table 19-M - Interpretation of sensitivity study results: MagPrep IV, PCR test kit A, *Enterobacteriaceae*

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

[1] ND: including PPND

Table 20-M - Interpretation of sensitivity study results: MagPrep IV, PCR test kit B, *Enterobacteriaceae*

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

[1] ND: including PPND

Table 21-S - Interpretation of sensitivity study results: StarPrep One Kit, PCR test kit A, *Cronobacter*

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	0	1	-1	3	1	6
2.2	3	1	2	3	4	6
3	0	0	0	3	0	6
4	0	0	0	3	0	6
All Categories (1, 2.1, 3, 4)	2	1	1	5	3	12
All Categories (1, 2.2, 3, 4)	5	1	4	5	6	12

[1] ND: including PPND

Table 22-S - Interpretation of sensitivity study results: StarPrep One Kit, PCR test kit B, *Cronobacter*

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	0	1	-1	3	1	6
2.2	2	1	1	3	3	6
3	0	0	0	3	0	6
4	0	0	0	3	0	6
All Categories (1, 2.1, 3, 4)	2	1	1	5	3	12
All Categories (1, 2.2, 3, 4)	4	1	3	5	5	12

[1] ND: including PPND

Table 21-M - Interpretation of sensitivity study results: MagPrep IV, PCR test kit A, *Cronobacter*

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	0	1	-1	3	1	6
2.2	2	1	1	3	3	6
3	1	0	1	3	1	6
4	1	0	1	3	1	6
All Categories (1, 2.1, 3, 4)	4	1	3	5	5	12
All Categories (1, 2.2, 3, 4)	6	1	5	5	7	12

[1] ND: including PPND

Table 22-M - Interpretation of sensitivity study results: MagPrep IV, PCR test kit B, *Cronobacter*

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	0	1	-1	3	1	6
2.2	2	1	1	3	3	6
3	0	0	0	3	0	6
4	1	0	1	3	1	6
All Categories (1, 2.1, 3, 4)	3	1	2	5	4	12
All Categories (1, 2.2, 3, 4)	5	1	4	5	6	12

[1] ND: including PPND

3.1.7 Conclusion sensitivity study

For *Enterobacteriaceae*, the observed values of ND-PD and ND+PD for the 4 individual categories and for all categories, for both alternative DNA extraction methods (StarPrepOne, MagPrep IV) and for **PCR test kit A and test kit B**, meet the acceptability limits (observed values ≤ AL).

For *Cronobacter*, the observed values of ND-PD and ND+PD for the 4 individual categories and for all categories, for both alternative DNA extraction methods (StarPrepOne, MagPrep IV) and for **PCR test kit A and test kit B,** 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 or two relevant target micro-organism for this item were chosen for each of the Categories in this validation study, as shown in Table 23.

Table 23 - 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	*Cronobacter dublinensis*	18705 D	Milk powder facility	at least 2 weeks at room temperature
		Salmonella Paratyphi B/Java	Salm 5F	Skim milk powder	at least 2 weeks at room temperature
Probiotic containing products	Infant fomula plus probiotics (*L.reuteri*)	*Cronobacter sakazakii*	NCTC 11467	Human	Lenticule based
		Salmonella Typhimurium	ATCC 14028	Chicken organs	Lenticule based
Ingredients	Starch	*Cronobacter muytjensii*	21870 D	WDCM 00213 (unknown)	at least 2 weeks at room temperature
		Salmonella Derby	NCTC 5722	Not stated	Lenticule based
Environmental samples	Vacuum cleaner residues	*Cronobacter turicensis*	18703 D	Human neonate	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 0,4 – 1,75 CFU per sample (20x)
- A higher level, which was inoculated with 2 – 10 CFU per sample (5x).

Test portions were individually inoculated and kept at an appropriate time/temperature for stabilization before actual testing (see Table 23).

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 24 for *Enterobacteriaceae* and in Tables 25 for *Cronobacter*.
Note that the data on the Item-Strain combination Starch-*Cronobacter* could only be used for evaluation on *Cronobacter*, not on *Enterobacteriaceae*, due to a natural contamination of the batch of starch with a *Pantoa* spp. strain. This strain was not detected by the alternative method during the RLOD study, but re-testing a pure culture of this isolated strain did show a positive result in the alternative method.
The original batch of vacuum cleaner residues was found to be natural positive for *Cronobacter* (all samples) and data could not be used for *Enterobacteriaceae* but only for *Salmonella* interpretation (reported separately in the LR39 study). A second batch of vacuum cleaner residues was inoculated with *Cronobacter* as scheduled and these data could be used for both *Enterobacteriaceae* and *Cronobacter* interpretation.

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

Table 24-S – Presentation of RLOD before and after confirmation of the alternative method results: StarPrep One Kit, PCR test kit A plus test kit B, *Enterobacteriaceae*

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 (Crono)	1	1,000	1,000	1,000	1,000
1	Infant formula (Salm)	1	1,000	1,000	1,000	1,000
2.1	Infant formula containing probiotics (Crono)	1	1,315	1,000	1,315	1,000
2.1	Infant formula containing probiotics (Salm)	1	1,000	1,000	1,000	1,000
2.2	Infant formula containing probiotics (Crono)	2	1,315	1,000	1,315	1,000
2.2	Infant formula containing probiotics (Salm)	2	0,872	0,872	0,872	0,872
3	Starch (Salm)	1	1,151	1,151	1,151	1,151
4	Vacuum cleaner residues (Crono)	1	1,120	1,120	1,120	1,120
Combined			*1,078*	*1,015*	*1,078*	*1,015*

Table 24-M – Presentation of RLOD before and after confirmation of the alternative method results: MagPrep IV, PCR test kit A plus test kit B, *Enterobacteriaceae*

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 (Crono)	1	1,000	1,000	1,000	1,000
1	Infant formula (Salm)	1	1,000	1,000	1,000	1,000
2.1	Infant formula containing probiotics (Crono)	1	1,000	0,828	1,000	0,828
2.1	Infant formula containing probiotics (Salm)	1	1,000	1,000	1,000	1,000
2.2	Infant formula containing probiotics (Crono)	2	0,698	0,698	0,698	0,698
2.2	Infant formula containing probiotics (Salm)	2	0,872	0,872	0,872	0,872
3	Starch (Salm)	1	1,151	1,151	1,151	1,151
4	Vacuum cleaner residues (Crono)	1	1,120	1,120	1,120	1,120
Combined			*0,985*	*0,971*	*0,985*	*0,971*

Table 25-S – Presentation of RLOD before and after confirmation of the alternative method results: StarPrep One Kit, PCR test kit A plus test kit B, *Cronobacter*

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	1,315	1,000	1,315	1,000
2.2	Infant formula containing probiotics	2	1,315	1,000	1,315	1,000
3	Starch	1	1,000	1,000	1,000	1,000
4	Vacuum cleaner residues	1	1,126	1,126	1,126	1,126
Combined			*1,123*	*1,023*	*1,123*	*1,023*

Table 25-M – Presentation of RLOD before and after confirmation of the alternative method results: MagPrep IV, PCR test kit A plus test kit B, *Cronobacter*

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	1,208	1,313	1,208	1,313
2.2	Infant formula containing probiotics	2	0,843	0,843	0,843	0,843
3	Starch	1	1,000	1,000	1,000	1,000
4	Vacuum cleaner residues	1	1,126	1,126	1,126	1,126
Combined			*1,025*	*1,056*	*1,025*	*1,056*

3.2.4 Conclusion RLOD study

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

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

3.3 Inclusivity/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

The Inclusivity and exclusivity 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. The relevant information as given here is taken over from the original validation study report (Jacobs-Reitsma et al. 2010).
Note: *E. sakazakii* nowadays *Cronobacter* spp.

Inclusivity:
53 pure cultures of *Enterobacteriaceae* strains relevant to the alternative method and the sample categories involved were selected for the inclusivity study on *Enterobacteriaceae*. Additionally, these strains also can be used within the exclusivity study on *E. sakazakii* (except for the 2 *E. sakazakii* strains within this group). The selected strains are listed in Annex I.
Overnight cultures of strains in BPW (18 ± 2h at 37°C) were diluted in peptone saline and added (1 ml) to fresh portions of 90 ml BPW and proceeded according to the specific protocols (for the alternative method only). No matrix was added.

50 pure cultures of *E. sakazakii* strains relevant to the alternative method and the sample categories involved were selected for the inclusivity study on *Cronobacter*. Additionally, these strains also can be used within the inclusivity study on *Enterobacteriaceae*. The selected strains are listed in Annex I.
Overnight cultures of strains in BPW (18 ± 2h at 37°C) were diluted in peptone saline and added (1 ml) to fresh portions of 90 ml BPW and proceeded according to the specific protocols (for the alternative method only). No matrix was added.

Exclusivity:
30 pure cultures of micro-organisms were selected both from strains known to cause interference with the target micro-organism and from strains naturally present in each sample category. The selected strains are listed in Annex I.

Overnight cultures of strains in BPW (18 ± 2h at 37°C) were diluted in peptone saline and added (1 ml) to fresh portions of 90 ml BPW and proceeded according to the specific protocols (for the alternative method only). No matrix was added.

All overnight BPW cultures were counted on spread plates of Nutrient Agar (NA).

3.3.2 Results inclusivity and exclusivity study

The raw data for from the original LR 8/9/19/20 study are given in Annex I.

All 53 *Enterobacteriaceae* strains gave a positive EB result with the alternative EB method. Additionally, also the 50 *E. sakazakii* strains all gave a positive EB result with the alternative EB method.

All 50 *E. sakazakii* strains gave a positive ES result with the alternative ES method. Also the 2 *E. sakazakii* strains EB14 and EB15 within the group of EB strains (Annex I) gave a positive ES result with the alternative ES method.

All 30 non-*Enterobacteriaceae* strains gave a negative EB and a negative ES result with the alternative EB and ES methods. Additionally, also the 51 *Enterobacteriaceae* but non-*E. sakazakii* strains (Annex I) all gave a negative ES result with the alternative ES method.

3.3.3 Conclusion inclusivity and exclusivity study

The alternative *Enterobacteriaceae* detection methods are selective and specific.

The alternative *Cronobacter* detection methods are selective and specific.

3.4 Conclusions Method Comparison Study

Overall, the conclusions for the Method Comparison Study are:

For *Enterobacteriaceae*, the observed values of ND-PD and ND+PD for the 4 individual categories and for all categories, for both alternative DNA extraction methods (StarPrepOne, MagPrep IV) and for PCR test kit A and test kit B, meet the acceptability limits (observed values ≤ AL).

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

The alternative *Enterobacteriaceae* detection methods are selective and specific.

For *Cronobacter*, the observed values of ND-PD and ND+PD for the 4 individual categories and for all categories, for both alternative DNA extraction methods (StarPrepOne, MagPrep IV) and for PCR test kit A and test kit B, meet the acceptability limits (observed values ≤ AL).

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

The alternative *Cronobacter* detection methods are 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 organization

4.1.1 General
The interlaboratory 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 the renewal studies. The relevant information as given here is taken over from the original study report (Jacobs-Reitsma et al., 2010).
The relevant parts of the existing interlaboratory study data were re-evaluated according to the ISO 16140-2 (2016) and the MVTC interpretation document (doc 2016-082 GC).
The original ILS was carried out with samples inoculated with an *Enterobacter cloaca* strain (*Enterobacteriaceae* detection part) as well as samples inoculated with a *Cronobacter sakazakii* strain (*Cronobacter* detection part). However, all *Enterobacter cloaca*-inoculated samples tested positive, so no fractional recovery level was obtained, which is nowadays preferred for evaluation under the new ISO 16140-2:2016. Therefore, the data obtained with the *Cronobacter*-inoculated samples were used for re-evaluation of the ILS for both the *Enterobacteriaceae* and the *Cronobacter* part of the study.

4.1.2 Collaborators number
A total of 20 collaborative laboratories from 5 different European countries (Annex O) have participated to the Interlaboratory Study. Labs participated, either using test kit A (7 labs) or test kit B (7 labs) or both test kits (6 labs).

4.1.3 Matrix and strain used
The matrix infant formula was inoculated with:
Spray-dried milk powder containing *Cronobacter sakazakii* strain ATCC 29544 (originating from a child's throat).

4.1.4 Samples
The original inoculum powders were "diluted" in blank infant formula in steps of 1 g in 10 g and thoroughly mixing per step to appropriate levels for individual inoculation of the L1 and L2 samples. Inoculum portions of 1 gram were individually added to each of pre-prepared 99 gram blank infant formula samples to obtain the various ILS samples.

4.1.5 Inoculation
Inoculation of the samples took place on Wednesday 2 September 2009 and samples were transported immediately to the 19 labs, to arrive on the following Thursday or Friday.
Intended contamination levels were:

L0 (EBES0)	blank	0 cfu/100 g
L1 (ES1)	low level *Enterobacteriaceae/Cronobacter*	5 cfu/100 g
L2 (ES2)	high level *Enterobacteriaceae/Cronobacter*	25 cfu/100 g

4.1.6 Labelling and shipping
The labs received blindly coded samples EBES 1 - EBES 40.
Transport of samples was under UN 3373 and in isolating boxes with ice packs. Temperature during transport was monitored by means of a temperature probe (iButton, http://www.maxim-ic.com/products/ibutton/) to be send back to the expert lab upon receipt of the samples.
The arrival time and the condition of the samples was recorded by each lab on the "sample receipt form" .

4.1.7 Analysis
The participating labs were asked to examine 40 dry infant formula samples of 100 gram each on presence of both *Enterobacteriaceae* (EB) and of *E. sakazakii* (ES) (nowadays: *Cronobacter sakazakii*), consisting of 8 replicates at 3 levels of the various contaminations, combining the blank samples for EB and ES.

Also, labs were asked to test 1 additional blank 10 g dried infant formula sample on presence of natural background flora by using ISO 4833 (pour plating in PCMA, 3 days at 30°C).

4.2 Experimental parameters controls

4.2.1 Contamination levels

The level of the powders that were used as the L1 and L2 inoculum was tested in 10-fold on Wednesday 2 September and on Monday 7 September 2009. 1 gram portions were diluted in 9 ml BPW and 5 ml of this 10-1 dilutions were pour-plated in VRBG (14 cm dishes). The calculated contamination levels and the sample codifications are given in Table 26.

Table 26 - Contamination levels of the coded samples

Level	Samples Coded EBES	Theoretical target level (cfu/100 g sample)	True level (cfu/100 g sample)	Low limit / (cfu/100 g sample	High limit / (cfu/100 g sample
L0	1, 11, 19, 20, 27, 35, 38, 40	blank	-	-	-
ES L1	3, 8, 10, 13, 16, 33, 34, 37	5	3,8	2,3	6,2
ES L2	5, 7, 15, 18, 21, 24, 8, 32	25	33,4	22,1	50,4

4.2.2 Logistic conditions

Upon receipt, each collaborative laboratory was requested to test the samples according to the instructions provided by the Expert Laboratory, starting on Monday 7 September 2009. Due to logistic problems with the delivery of the media, some labs had to delay their start of analysis to Tuesday 8 September 2009. In addition, the Expert Lab performed the total analysis again on a spare set of samples on Tuesday 8 September 2009. No adverse effects were noticed, so all data could be entered into the evaluation process.

The date and time of arrival of the samples, as well as the temperature recordings from the iButtons and the date of staring the analysis are given in Table 27.

Table 27- Sample transport data, starting date of the analysis and TVC results

Lab code	Date of arrival	Time of arrival	Temp iButton (°C)			Date of starting the analysis	TVC (in cfu/g)
			upon arrival	minimum during transport	maximum during transport		
A	3-9-2009	14 h	6,0	3,5	10,0	**8-9-2009**	<10
B	**4-9-2009**	16 h	15,0	6,0	15,0	7-9-2009	<100
C	3-9-2009	9.15 h	12,0	6,5	12,0	7-9-2009	<100
D	3-9-2009	13 h	12,5	7,5	13,0	7-9-2009	8,4E+03
E	**4-9-2009**	13 h	13,0	5,5	13,0	7-9-2009	<10
F	3-9-2009	12 h	8,5	8,0	13,0	7-9-2009	<100
G	**7-9-2009**	13.15 h	6,5	-1,5	6,5	7-9-2009	<100
H	3-9-2009	11.45 h	2,5	2,0	5,0	7-9-2009	<100
I	3-9-2009	14 h	11,0	5,0	11,0	7-9-2009	<10
J	3-9-2009	12.15 h	1,5	-9,5	1,5	7-9-2009	<10
K	3-9-2009	14 h	10,5	4,5	10,5	7-9-2009	<10
L	3-9-2009	7.15 h	9,0	8,0	10,0	7-9-2009	<10
M	3-9-2009	14.45 h	5,5	3,0	6,5	7-9-2009	1,3E+03
N	3-9-2009	11.30 h	4,0	-0,5	4,0	7-9-2009	<10
O	3-9-2009	11 h	4,0	3,0	5,5	7-9-2009	<100
P	3-9-2009	14 h	13,5	3,0	15,5	7-9-2009	<100
Q	3-9-2009	12.15 h	13,5	7,0	13,5	7-9-2009	<10
R	3-9-2009	15.30 h	6,0	4,5	6,0	7-9-2009	<10
S	**4-9-2009**	13 h	16,0	7,5	16,5	**8-9-2009**	1,4E+03
T	3-9-2009	11.30 h	6,5	6,5	11,5	7-9-2009	<10
EL	3-9-2009	12 h	17,5	9,0	17,5	7-9-2009	<100

4.3 Calculation and summary of data

The ES (*Cronobacter*) part of the study did show fractional recovery at the L1 inoculation level, and was used for interpretation of results for detection of *Enterobacteriaceae* as well as for interpretation of results for detection of *Cronobacter*.
The relevant raw data per participating laboratory are given in Annex P.

4.3.1 MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 28. Identical results were obtained for the sample set starting the analysis on 7-9-2009 and the samples set starting the analysis on 8-9-2009.

Table 28 – Results obtained by the expert laboratory

Target	Level	Reference method	Alternative method	
			PCR kit A	PCR kit B
Enterobacteriaceae	L0	0/8	0/8	0/8
Enterobacteriaceae	L1	7/8	7/8	7/8
Enterobacteriaceae	L2	8/8	8/8	8/8
Cronobacter	L0	0/8	0/8	0/8
Cronobacter	L1	7/8	7/8	7/8
Cronobacter	L2	8/8	8/8	8/8

4.3.2 Results obtained by the collaborative laboratories

- *Mesophilic aerobic flora enumeration*

Depending on the Lab results, the enumeration levels varied from <10 to 8,4 x 10^3 cfu/g (Table 27).

- *Enterobacteriaceae detection*

20 collaborators participated to the study (Annex O, Annex P). The results obtained by the individual collaborators in the interlaboratory study are summarised in Table 29 (reference method) and Table 30 (alternative method, PCR kit A and PCR kit B).

- *Cronobacter detection*

20 collaborators participated to the study (Annex O, Annex P). The results obtained by the individual collaborators in the interlaboratory study are summarised in Table 31 (reference method) and Table 32 (alternative method, PCR kit A and PCR kit B).

Table 29 - Positive results *Enterobacteriaceae* by the Reference method (ALL the collaborators)

Laboratory	(PCR kit A)			(PCR kit B)		
	Contamination Level			Contamination Level		
	L0	L1	L2	L0	L1	L2
Lab A				0	6	8
Lab B				0	6	8
Lab C				0	4	8
Lab D	0	5	8			
Lab E				1	3	8
Lab F				0	3	8
Lab G	0	4	8			
Lab H				0	5	8
Lab I	0	7	8	0	7	8
Lab J	1	5	8			
Lab K	0	6	8	0	6	8
Lab L				0	6	8
Lab M	0	3	8	0	3	8
Lab N	0	8	8			
Lab O	0	6	8			
Lab P	0	6	8			
Lab Q	0	3	8	0	3	8
Lab R	2	5	7	2	5	7
Lab S	0	6	8			
Lab T	0	6	8	0	6	8
Total	**3**	**70**	**103**	**3**	**63**	**103**
	P0	P1	P2	P0	P1	P2

Table 30 - Positive results *Enterobacteriaceae* (before and after confirmation) by the Alternative methods kit A and kit B (ALL the collaborators)

Laboratory	PCR kit A						PCR kit B					
	Contamination Level						Contamination Level					
	L0		L1		L2		L0		L1		L2	
	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.
Lab A							0	0	6	6	8	8
Lab B							0	0	6	6	8	8
Lab C							0	0	4	4	8	8
Lab D	0	0	5	5	8	8						
Lab E							0	0	3	3	8	8
Lab F							0	0	3	3	8	8
Lab G	2	0	4	4	8	8						
Lab H							0	0	5	5	8	8
Lab I	0	0	7	7	8	8	0	0	7	7	8	8
Lab J	1	1	5	5	8	8						
Lab K	2	2	6	6	8	8	0	0	6	6	8	8
Lab L							0	0	6	6	8	8
Lab M	0	0	3	3	8	8	0	0	3	3	8	8
Lab N	0	0	8	8	8	8						
Lab O	2	0	6	6	8	8						
Lab P	3	0	6	6	8	8						
Lab Q	0	0	3	3	8	8	1	0	3	3	8	8
Lab R	8	2	8	5	8	7	7	2	8	5	8	7
Lab S	0	0	6	6	8	8						
Lab T	0	0	6	6	8	8	0	0	6	6	8	8
Total	**18**	**5**	**73**	**70**	**104**	**103**	**8**	**2**	**66**	**63**	**104**	**103**
	P0	CP0	P1	CP1	P2	CP2	P0	CP0	P1	CP1	P2	CP2

Table 31 - Positive results by the *Cronobacter* Reference method (ALL the collaborators)

Laboratory	(PCR kit A)			(PCR kit B)		
	Contamination Level			Contamination Level		
	L0	L1	L2	L0	L1	L2
Lab A				0	6	8
Lab B				0	5	8
Lab C				0	4	8
Lab D	0	5	8			
Lab E				0	3	8
Lab F				0	3	8
Lab G	0	4	8			
Lab H				0	5	8
Lab I	0	7	8	0	7	8
Lab J	0	6	8			
Lab K	0	6	8	0	6	8
Lab L				0	6	8
Lab M	0	3	8	0	3	8
Lab N	0	8	8			
Lab O	0	6	8			
Lab P	0	6	8			
Lab Q	0	3	8	0	3	8
Lab R	1	5	6	1	5	6
Lab S	0	6	8			
Lab T	0	6	8	0	6	8
Total	**1**	**71**	**102**	**1**	**62**	**102**
	P0	P1	P2	P0	P1	P2

Table 32 - Positive results *Cronobacter* (before and after confirmation) by the Alternative methods kit A and kit B (ALL the collaborators)

Laboratory	PCR kit A						PCR kit B					
	Contamination Level						Contamination Level					
	L0		L1		L2		L0		L1		L2	
	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.
Lab A							0	0	6	6	8	8
Lab B							0	0	5	5	8	8
Lab C							0	0	4	4	8	8
Lab D	0	0	5	5	8	8						
Lab E							0	0	3	3	8	8
Lab F							0	0	3	3	8	8
Lab G	2	0	4	4	8	8						
Lab H							0	0	5	5	8	8
Lab I	0	0	7	7	8	8	0	0	7	7	8	8
Lab J	0	0	5	5	7	7						
Lab K	1	1	6	6	8	8	0	0	6	6	8	8
Lab L							0	0	6	6	8	8
Lab M	0	0	3	3	8	8	0	0	3	3	8	8
Lab N	0	0	8	8	8	8						
Lab O	1	0	6	6	8	8						
Lab P	1	0	6	6	8	8						
Lab Q	0	0	3	3	8	8	0	0	3	3	8	8
Lab R	7	1	7	4	8	6	5	1	7	4	8	6
Lab S	0	0	6	6	8	8						
Lab T	0	0	6	6	8	8	0	0	6	6	8	8
Total	**12**	**2**	**72**	**69**	**103**	**101**	**5**	**1**	**64**	**61**	**104**	**102**
	P0	CP0	P1	CP1	P2	CP2	P0	CP0	P1	CP1	P2	CP2

4.3.3 Results of the collaborators retained for interpretation

The *Enterobacteriaceae* results obtained with the 11 collaborators (test kit A) and 10 collaborators (test kit B) kept for interpretation are presented in Table 33 (reference method) and Table 34 (alternative methods).

Lab R encountered many problems in several aspects of the study and was therefore excluded from the interpretation. Lab E and Lab K were excluded from the interpretation due to the false-positive blanks that these labs reported in either the reference or the alternative method. Lab J also reported a blank sample positive for *Enterobacteriaceae*, but this was a consistent result for both the reference and the alternative method, and this may just have been a very rare naturally contaminated sample for *Enterobacteriaceae*, though the strain was not available anymore for further investigation.

The *Cronobacter* results obtained with the11 collaborators (test kit A) and 11 collaborators (test kit B) kept for interpretation are presented in Table 35 (reference method) and Table 36 (alternative methods).

Lab R encountered many problems in several aspects of the study and was therefore excluded from the interpretation. Lab K was excluded from the interpretation due to the false-positive blank reported in the alternative method.

Table 33 - Positive *Enterobacteriaceae* results by the Reference method (Without Labs E, K, R)

Laboratory	(PCR kit A)			(PCR kit B)		
	Contamination Level			Contamination Level		
	L0	L1	L2	L0	L1	L2
Lab A				0	6	8
Lab B				0	6	8
Lab C				0	4	8
Lab D	0	5	8			
Lab F				0	3	8
Lab G	0	4	8			
Lab H				0	5	8
Lab I	0	7	8	0	7	8
Lab J	1	5	8			
Lab L				0	6	8
Lab M	0	3	8	0	3	8
Lab N	0	8	8			
Lab O	0	6	8			
Lab P	0	6	8			
Lab Q	0	3	8	0	3	8
Lab S	0	6	8			
Lab T	0	6	8	0	6	8
Total	**1**	**59**	**88**	**0**	**49**	**80**
	P0	P1	P2	P0	P1	P2

Table 34 – Positive *Enterobacteriaceae* results (before and after confirmation) by the Alternative methods kit A and kit B (Without Labs E, K, R)

Laboratory	PCR kit A						PCR kit B					
	Contamination Level						Contamination Level					
	L0		L1		L2		L0		L1		L2	
	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.
Lab A							0	0	6	6	8	8
Lab B							0	0	6	6	8	8
Lab C							0	0	4	4	8	8
Lab D	0	0	5	5	8	8						
Lab F							0	0	3	3	8	8
Lab G	2	0	4	4	8	8						
Lab H							0	0	5	5	8	8
Lab I	0	0	7	7	8	8	0	0	7	7	8	8
Lab J	1	1	5	5	8	8						
Lab L							0	0	6	6	8	8
Lab M	0	0	3	3	8	8	0	0	3	3	8	8
Lab N	0	0	8	8	8	8						
Lab O	2	0	6	6	8	8						
Lab P	3	0	6	6	8	8						
Lab Q	0	0	3	3	8	8	1	0	3	3	8	8
Lab S	0	0	6	6	8	8						
Lab T	0	0	6	6	8	8	0	0	6	6	8	8
Total	**8**	**1**	**59**	**59**	**88**	**88**	**1**	**0**	**49**	**49**	**80**	**80**
	P0	CP0	P1	CP1	P2	CP2	P0	CP0	P1	CP1	P2	CP2

Table 35 - Positive *Cronobacter* results by the reference method (Without Labs K and R)

Laboratory	(PCR kit A)			(PCR kit B)		
	Contamination Level			Contamination Level		
	L0	L1	L2	L0	L1	L2
Lab A				0	6	8
Lab B				0	5	8
Lab C				0	4	8
Lab D	0	5	8			
Lab E				0	3	8
Lab F				0	3	8
Lab G	0	4	8			
Lab H				0	5	8
Lab I	0	7	8	0	7	8
Lab J	0	6	8			
Lab L				0	6	8
Lab M	0	3	8	0	3	8
Lab N	0	8	8			
Lab O	0	6	8			
Lab P	0	6	8			
Lab Q	0	3	8	0	3	8
Lab S	0	6	8			
Lab T	0	6	8	0	6	8
Total	**0**	**60**	**88**	**0**	**51**	**88**
	P0	P1	P2	P0	P1	P2

Table 36 – Positive *Cronobacter* results (before and after confirmation) by the alternative methods (Without Labs K and R)

Laboratory	PCR kit A						PCR kit B					
	Contamination Level						Contamination Level					
	L0		L1		L2		L0		L1		L2	
	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.	Before confirm.	After confirm.
Lab A							0	0	6	6	8	8
Lab B							0	0	5	5	8	8
Lab C							0	0	4	4	8	8
Lab D	0	0	5	5	8	8						
Lab E							0	0	3	3	8	8
Lab F							0	0	3	3	8	8
Lab G	2	0	4	4	8	8						
Lab H							0	0	5	5	8	8
Lab I	0	0	7	7	8	8	0	0	7	7	8	8
Lab J	0	0	5	5	7	7						
Lab L							0	0	6	6	8	8
Lab M	0	0	3	3	8	8	0	0	3	3	8	8
Lab N	0	0	8	8	8	8						
Lab O	1	0	6	6	8	8						
Lab P	1	0	6	6	8	8						
Lab Q	0	0	3	3	8	8	0	0	3	3	8	8
Lab S	0	0	6	6	8	8						
Lab T	0	0	6	6	8	8	0	0	6	6	8	8
Total	**4**	**0**	**59**	**59**	**87**	**87**	**0**	**0**	**51**	**51**	**88**	**88**
	P0	CP0	P1	CP1	P2	CP2	P0	CP0	P1	CP1	P2	CP2

4.3.4 Calculation of the specificity percentage (SP)

The percentage specificities (SP) of the reference method and of the alternative methods Kit A and Kit B, using the data after confirmation, based on the results of level L0 are given in Table 37 for *Enterobacteriaceae* and in Table 38 for *Cronobacter*.

.

Table 37 - Percentage specificity *Enterobacteriaceae*

Specificity for the reference method (Kit A part)	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	99 %
Specificity for the alternative method Kit A	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	99 %
Specificity for the reference method (Kit B part)	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) x\ 100\ \% =$	100 %
Specificity for the alternative method Kit B	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) x\ 100\ \% =$	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

Table 38 - Percentage specificity *Cronobacter*

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

4.3.5 Calculation of the sensitivity for the alternative method (SE_{alt}), the sensitivity for the reference method (SE_{ref}), the relative trueness (RT) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for:
the low (L1) inoculation level *Enterobacteriaeceae*, Kit A and Kit B.
the low (L1) inoculation level *Cronobacter*, Kit B.
the low (L1) and high (L2) inoculation level *Cronobacter*, Kit A.

These inoculation levels were 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, and Level 2 if applicable, is provided in Table 39 for *Enterobacteriaceae* and in Table 40 for *Cronobacter*.

Table 39 - Summary of the obtained results with the reference method and the alternative method, *Enterobacteriaceae*

Level	Kit	Response	Reference method positive (R+)	Reference method negative (R-)
1	Kit A	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 59**	Positive deviation (R-/A+) **PD = 0**
		Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 29**
1	Kit B	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 49**	Positive deviation (R-/A+) **PD = 0**
		Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 31**

Table 2 - Summary of the obtained results with the reference method and the alternative method, *Cronobacter*

Level	Kit	Response	Reference method positive (R+)	Reference method negative (R-)
1	Kit A	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 59**	Positive deviation (R-/A+) **PD = 0**
		Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 1**	Negative agreement (A-/R-) **NA = 28**
2	Kit A	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 87**	Positive deviation (R-/A+) **PD = 0**
		Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 1**	Negative agreement (A-/R-) **NA = 0**
1	Kit B	**Alternative method positive (A+)**	Positive agreement (A+/R+) **PA = 51**	Positive deviation (R-/A+) **PD = 0**
		Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 37**

Based on the data summarized in Table 39 and Table 40, 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 41 (*Enterobacteriaceae*) and Table 42 (*Cronobacter*).

Table 41 - Sensitivity, relative trueness and false positive ratio percentages, *Enterobacteriaceae*

		Kit A Level 1	Kit B Level 1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} x\ 100\% =$	100%	100%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} x\ 100\% =$	100%	100%
Relative trueness	$RT = \frac{(PA+NA)}{N} x\ 100\% =$	100%	100%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} x\ 100\% =$	24,1%	3,2%

Table 42 - Sensitivity, relative trueness and false positive ratio percentages, *Cronobacter*

		Kit A Level 1	Kit A Level 2	Kit B Level 1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} x\ 100\% =$	98,3%	98,9%	100%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} x\ 100\% =$	100%	100%	100%
Relative trueness	$RT = \frac{(PA+NA)}{N} x\ 100\% =$	98,9%	98,9%	100%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} x\ 100\% =$	14,3%	/	0%

4.3.6 Interpretation of data

No positive deviations were observed. The two negative deviations, *Cronobacter* only, are listed in Table 43.

Table 43 - Negative deviations observed in the ILS

LAB J		**Reference methods results**		**Alternative methods results**					
				KIT A, hybridization probes					
		Confirmed detection of		LC 2.0					
				Enterobacteriaceae			*Cronobacter*		
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result
L1	EBES 34	-	+	-	-	NA	-	-	ND
L2	EBES 32	+	+	+	+	PA	-	-	ND

*after culture-confirmation

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 (so L_1 and possibly L_2). The observed value found for (ND – PD) and (ND + PD) shall not be higher than the AL.
The interpretation of the data is given in Table 44.

Table 44 – Interpretation of the interlaboratory study results

Target	Kit	Level	Negative Deviations (ND)	Positive deviations (PD)	ND-PD	Acceptability Limit (AL)	ND+PD	Acceptability Limit (AL)	Conclusion
EB	Kit A	L1	0	0	0	4	0	4	Accepted
EB	Kit B	L1	0	0	0	3	0	4	Accepted
Crono	Kit A	L1	1	0	1	4	1	4	Accepted
Crono	Kit A	L2	1	0	1	4	1	4	Accepted
Crono	Kit B	L1	0	0	0	4	0	4	Accepted

4.3.7 Conclusion Interlaboratory Study

The observed values for ND-PD and ND+PD meet the acceptability limits (observed values ≤ AL).
This is valid for: *Enterobacteriaeceae*, Kit A and Kit B; *Cronobacter*, Kit A and Kit B.

4.3.8 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 45).

Table 45 – RLOD calculations interlaboratory study data

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
EB Kit A, 11 labs	1,000	0,696	1,437	0,000	0,181	0,000	1,000
EB Kit B, 10 labs	1,000	0,675	1,481	0,000	0,196	0,000	1,000
Crono, Kit A, 11 labs	1,110	0,784	1,571	0,104	0,174	0,599	0,549
Crono Kit B, 11 labs	1,000	0,682	1,466	0,000	0,191	0,000	1,000

5 CONCLUSION

The **method comparison study conclusions** are:

For *Enterobacteriaceae*, the observed values of ND-PD and ND+PD for the 4 individual categories and for all categories, for both alternative DNA extraction methods (StarPrepOne, MagPrep IV) and for PCR test kit A and test kit B, meet the acceptability limits (observed values ≤ AL).

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

The alternative *Enterobacteriaceae* detection methods are selective and specific.

For *Cronobacter*, the observed values of ND-PD and ND+PD for the 4 individual categories and for all categories, for both alternative DNA extraction methods (StarPrepOne, MagPrep IV) and for PCR test kit A and test kit B, meet the acceptability limits (observed values ≤ AL).

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

The alternative *Cronobacter* detection methods are selective and specific.

The **interlaboratory study conclusions** are:

The observed values for ND-PD and ND+PD meet the acceptability limits (observed values ≤ AL). This is valid for: *Enterobacteriaeceae*, Kit A and Kit B; *Cronobacter*, Kit A and Kit B.

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

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® StarPrep One Kit for manual DNA extraction, shows comparable performance to the reference method EN-ISO 21528-1:2017 for the detection of *Enterobacteriaceae* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® StarPrep One Kit for manual DNA extraction, shows comparable performance to the reference method EN-ISO 22964:2017 for the detection of *Cronobacter* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® Magnetic Preparation Kit IV forsemi-automated DNA extraction, shows comparable performance to the reference method EN-ISO 21528-1:2017 for the detection of *Enterobacteriaceae* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The alternative method foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit (Kit A: Hybridization probes and Kit B: 5'Nuclease), using the foodproof® Magnetic Preparation Kit IV for semi-automated DNA extraction, shows comparable performance to the reference method EN-ISO 22964:2017 for the detection of *Cronobacter* in Infant formula and infant cereals, Probiotics containing products, Ingredients, and Environmental samples.

The overview on the claims for the current LR8/9/19/20 certificate is given in the Table below:

Detection of *Enterobacteriacea* and/or detection of *Cronobacter* spp.

	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 S 400 07						Automated protocol **food**proof MagPrep IV Kit S 400 15					
	Real-time PCR	**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			
	Thermocyclers	LC 480 II	LC 2.0	LC 480 II	IQ5	ABI 7500	Mx 3005	LC 480 II	LC 2.0	LC 480 II	IQ5	ABI 7500	Mx 3005
	Software version	1.5.1	4.1	1.5.1	2.0	2.0.6	MX4.1d	1.5.1	4.1	1.5.1	2.0	2.0.6	MX4.1d
Scope (including subcultivation Protocol)	Infant formula and infant cereals (P1)	X	X	X	X	X	X	X	X	X	X	X	X
	Probiotics containing products (P1)	X	X	X	X	X	X	X	X	X	X	X	X
	Probiotics containing products (P2)	X	X	X	X	X	X	X	X	X	X	X	X
	Ingredients (P1)	X	X	X	X	X	X	X	X	X	X	X	X
	Environmental samples (P1)	X	X	X	X	X	X	X	X	X	X	X	X

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

6 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 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 21528-1:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection and enumeration by MPN technique with pre-enrichment

ISO 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection of *Enterobacteriaceae*

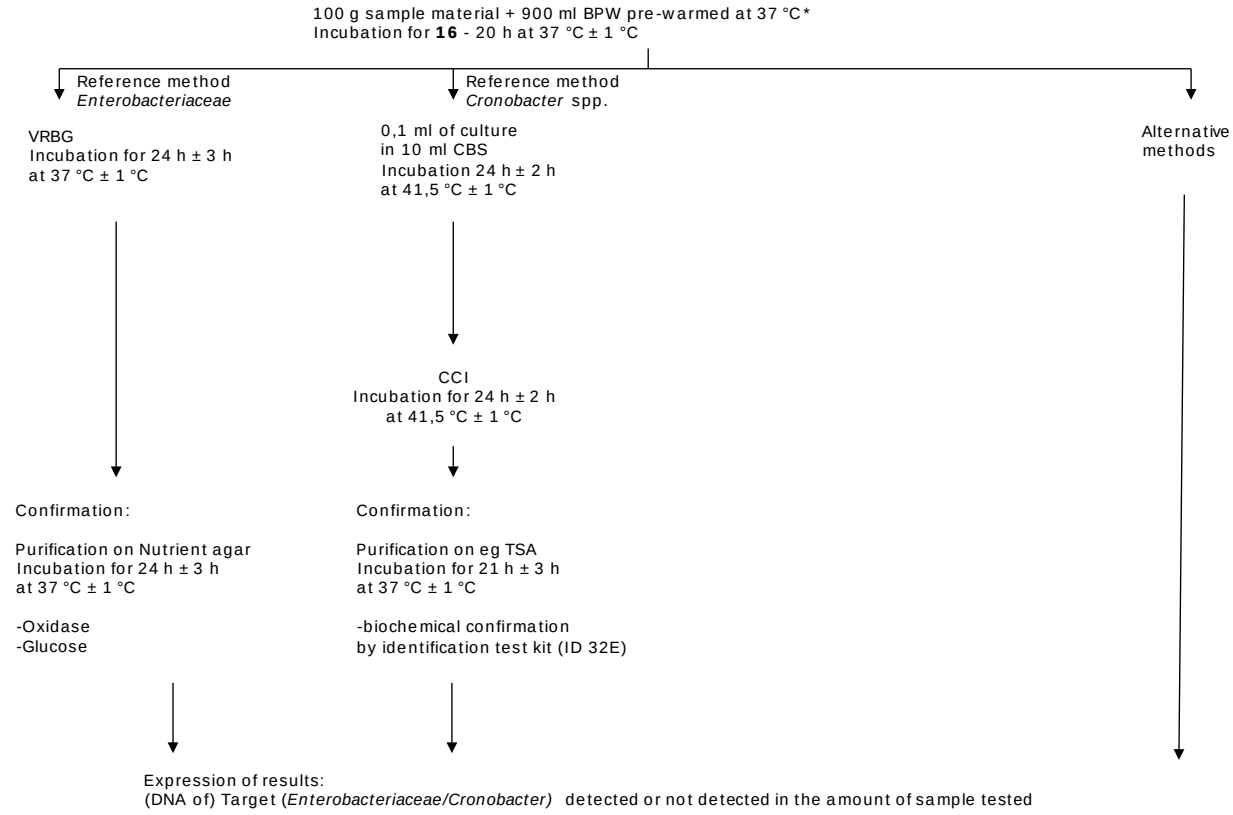
ISO/TS 22964:2006 Milk and milk products - Detection of *Enterobacter sakazakii*

ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

Jacobs-Reitsma, W.F., A. van Hoek, W. van Overbeek, E. Bouw, P. Boleij, C. Kandhai, and H. van der Voet. 2010. Expert Lab Report on the ISO 16140:2003 Validation of - the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection System, -Hybridization probes- (Art. No. R 300 15) and - the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit, -5'Nuclease- (Art. No. R 302 15). RIKILT confidential report R2010.506.

Jacobs-Reitsma, W.F., R. Diddens, E.M. Bouw, A. Gritchina, and W. van Overbeek. 2018. Extension Report for MicroVal certificate 2009 LR8/9/19/20, concerning the **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kits (BIOTECON Diagnostics Cat. No. R 310 27 and R 302 27) for detection of *Enterobacteriaceae* and/ or *Cronobacter* spp. in infant formula and infant cereals, probiotics containing products, ingredients and environmental samples. RIVM-Z&O Confidential Letter-Report 00148/2018.

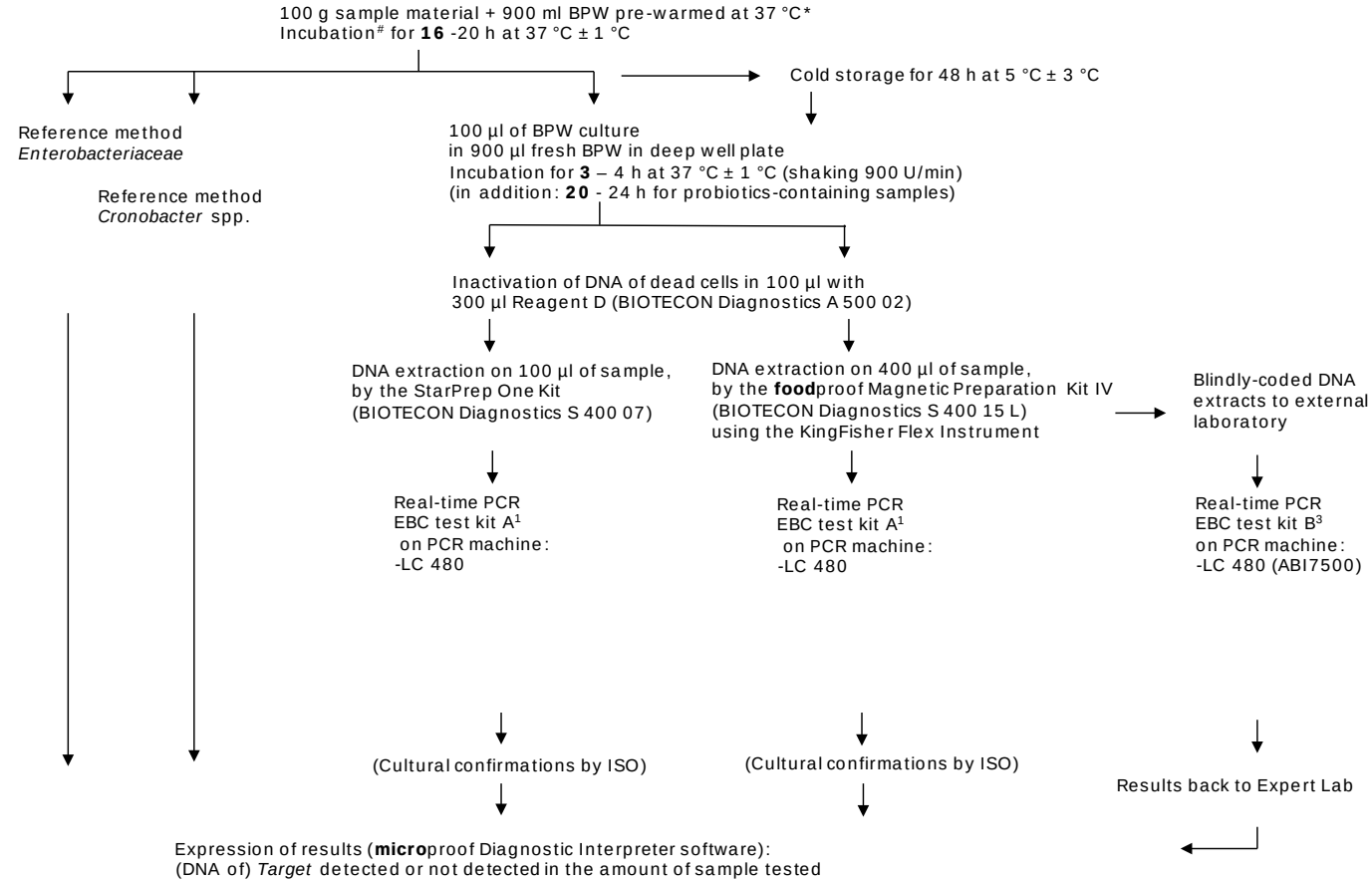
Annex A: Flow diagram of the reference methods



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

Pre-enrichment 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: Flow diagram of the alternative methods



*Some sample types are tested according to an adjusted pre-enrichment protocol, see Introduction and Annex B.
All incubations are done according to the *minimum* incubation time (indicated in bold).
[1] EB/C test kit A: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - Hybridization probes - (BIOTECON Diagnostics R 310 15)

[3] EB/C test kit B: **food**proof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - 5'Nuclease - (BIOTECON Diagnostics R 302 15)

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, eg 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
Probiotic infant formula (*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
Probiotic infant formula (*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
Probiotic infant cereals (*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
Probiotic ingredients 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
Probiotic ingredients 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. 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 - Annex H Test kit inserts

For practical and ecological 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

The following kit inserts are available as separate pdf's.

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).

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

MICROVAL® NEN

Annex I. Inclusivity/exclusivity data from the original LR 8/9/19/20 studies

Annex XII Raw data inclusivity study *Enterobacteriaceae*

							kit A		kit B		
							EB	ES	EB	ES	
Sample nr.	Strain	Ref. nr.	Origin	Source	Date of DNA isolation	Inoculation per 90 ml BPW (cfu)	LC 2.0	LC 2.0	IQ5	IQ5	Remarks
EB1	*Budvicia aquatica*	Micr 553	Drinking water, Type strain		24-9-2008	291	pos*	neg	pos	neg	* result of the second test run (first run gave repetition)
EB2	*Citrobacter braakii*	Micr 354		Cheese	17-9-2008	165	pos	neg	pos	neg	
EB3	*Citrobacter freundii*	Micr 353		Food	17-9-2008	400	pos	neg	pos	neg	
EB4	*Citrobacter koseri*	Micr 373	Korea	Milk powder	17-9-2008	391	pos	neg	pos	neg	
EB5	*Citrobacter sedlakii*	Micr 371	UK	Food	17-9-2008	382	pos	neg	pos	neg	
EB6	*Edwardsiella tarda*	Micr 599	DSM 30052 (NCTC 10396)		14-1-2009	1455	pos	neg	pos	neg	
EB7	*Enterobacter intermedius*	Micr 547	Type strain	Surface water	17-9-2008	138	pos	neg	pos	neg	
EB8	*Enterobacter aerogenes*	Micr 534	Type strain	Sputum	17-9-2008	41	pos	neg	pos	neg	
EB9	*Enterobacter amnigenus*	Micr 370	UK	Food	17-9-2008	75	pos	neg	pos	neg	
EB10	*Enterobacter cloacae*	Micr 361		Infant formula	24-9-2008	1236	pos	neg	pos	neg	
EB11	*Enterobacter cloacae*	Micr 364	India	Environment: milk powder	24-9-2008	509	pos	neg	pos	neg	
EB51	*Enterobacter gergovia*	Micr 603	The Netherlands	Environment: sponge	14-1-2009	409	pos	neg	pos	neg	
EB52	*Enterobacter gergovia*	Micr 602	DSM 9245		14-1-2009	736	pos	neg	pos	neg	
EB12	*Enterobacter helveticus*	Micr 363	Switzerland	Environment: milk powder	24-9-2008	98	pos	neg	pos	neg	
EB53	*Enterobacter helveticus*	Micr 604		Milk powder	14-1-2009	364	pos	neg	pos	neg	
EB13	*Enterobacter hormaechei*	Micr 375	India	Milk powder	24-9-2008	1055	pos	neg	pos	neg	
EB14	*Enterobacter sakazakii*	Micr 372	Indonesia	Milk powder	24-9-2008	536	pos	pos	pos	pos	
EB15	*Enterobacter sakazakii*	Micr 374	France	Infant formula	24-9-2008	709	pos	pos	pos	pos	
EB16	*Enterobacter turicensis*	Micr 526	Switzerland, Type strain	Fruit powder	24-9-2008	673	pos	neg	pos	neg	
EB17	*Erwinia carotovora*	Micr 538	Denmark, Type strain	Potato	24-9-2008	58	pos	neg	pos	neg	
EB18	*Escherichia blattae*	Micr 558	Type strain	Hindgut of cockroach	24-9-2008	182	pos	neg	pos	neg	
EB19	*Escherichia coli*	Micr 358		Cheese	24-9-2008	909	pos	neg	pos	neg	
EB20	*Escherichia coli*	Micr 396	The Netherlands	Mussels	2-10-2008	125	pos	neg	pos	neg	
EB21	*Escherichia coli*	Micr 397	The Netherlands	Cockles	2-10-2008	96	pos	neg	pos*	neg	* result of the second test run (first run gave repetition)
EB22	*Escherichia coli*	Micr 387	ATCC 25922		2-10-2008	240	pos	neg	pos	neg	
EB23	*Escherichia hermanii*	Micr 365	Germany	Environment: milk powder	2-10-2008	225	pos	neg	pos	neg	
EB24	*Hafnia alvei*	Micr 356		Cosmetic: shampoo	2-10-2008	177	pos	neg	pos	neg	
EB25	*Hafnia alvei*	Micr 537	Type strain		2-10-2008	364	pos	neg	pos	neg	
EB26	*Klebsiella oxytoca*	Micr 355		Cheese	2-10-2008	141	pos	neg	pos	neg	
EB27	*Klebsiella pneumoniae*	Micr 359	The Netherlands	Environment: milk powder	2-10-2008	106	pos	neg	pos*	neg	* result of the second test run (first run gave repetition)
EB28	*Kluyvera cryocrescens*	Micr 549	Type strain	Human	2-10-2008	74	pos	neg	pos	neg	
EB29	*Pantoea agglomerans*	Micr 540	Type strain	Knee laceration	2-10-2008	51	pos	neg	pos	neg	
EB30	*Pantoea dispersa*	Micr 535	Japan, Type strain	Soil	2-10-2008	55	pos	neg	pos	neg	
EB31	*Proteus mirabilis*	Micr 357		Cosmetic: shampoo	9-10-2008	382	pos	neg	pos*	neg	* result of the second test run (first run gave repetition)
EB32	*Proteus vulgaris*	Micr 530		Inner ear infection	9-10-2008	218	pos	neg	pos	neg	
EB33	*Providencia alcalifaciens*	Micr 536	Serovar 019:H2, Type strain	Faeces	9-10-2008	55	pos	neg	pos	neg	
EB34	*Providencia rettgeri*	Micr 600	DSM 1131 (NCTC 7481)		14-1-2009	273	pos	neg	pos	neg	
EB35	*Providencia stuartii*	Micr 544	USA, Type strain	Human	9-10-2008	273	pos	neg	pos*	neg	* result of the second test run (first run gave repetition)
EB36	*Rahnella aquatilis*	Micr 550		Drinking water	9-10-2008	8	pos	neg	pos	neg	
EB37	*Raoultella terrigena*	Micr 531	Type strain	Drinking water	9-10-2008	62	pos	neg	pos	neg	
EB38	*Salmonella Enteritidis*	Micr 706		Surface water	9-10-2008	364	pos	neg	pos	neg	
EB39	*Salmonella Infantis*	Micr 691		Milk powder	9-10-2008	138	pos	neg	pos	neg	
EB40	*Salmonella Panama*	Micr 701		Infant formula	9-10-2008	755	pos	neg	pos	neg	
EB41	*Salmonella Typhimurium*	Micr 708		Raw milk cheese	9-10-2008	273	pos	neg	pos	neg	
EB42	*Serratia ficaria*	Micr 362	Germany	Environment: milk powder	9-10-2008	143	pos	neg	pos	neg	
EB43	*Serratia fonticola*	Micr 546	Type strain	Water	9-10-2008	373	pos	neg	pos	neg	
EB44	*Serratia marcescens*	Micr 240	ATCC 274		9-10-2008	98	pos	neg	pos	neg	
EB45	*Serratia proteamaculans*	Micr 543	Ireland	Milk	9-10-2008	455	pos	neg	pos	neg	
EB46	*Shigella boydii*	Micr 556	India, Serovar 2, Type strain		9-10-2008	8	pos	neg	pos	neg	
EB47	*Shigella flexneri*	Micr 368	ATCC 12022		14-1-2009	627	pos	neg	pos	neg	
EB48	*Shigella sonnei*	Micr 598	DSM 5570 (ATCC 29930)		14-1-2009	936	pos	neg	pos	neg	
EB49	*Yersinia aldovae*	Micr 483	Czechoslovakia	Drinking water	2-2-2009	873	pos*	neg	pos*	neg	* result after re-culture of strain (first/second runs gave repetitions)
EB50	*Yersinia enterocolitica*	Micr 369	ATCC 9610		14-1-2009	674	pos	neg	pos	neg	

MICROVAL® NEN

Annex I. Inclusivity and exclusivity data from the original LR 8/9/19/20 studies, continued.

Annex XIII Raw data inclusivity study *E. sakazakii*

							kit A		kit B		
							EB	ES	EB	ES	
Sample nr.	Strain	Ref. nr.	Origin	Source	Date of DNA isolation	Inoculation per 90 ml BPW (cfu)	LC 2.0	LC 2.0	IQ5	IQ5	Remarks
ES1	*Cronobacter dublinensis subsp.*	Micr 528	DSM 18705, Type strain	Milk powder production facility	17-9-2008	105	pos	pos	pos	pos	
ES2	*Cronobacter muytjensii*	Micr 485	ATCC 51329, Type strain	unknown	17-9-2008	300	pos	pos	pos	pos	
ES3	*Cronobacter turicensis*	Micr 527	DSM 18703, Type strain	Human neonate, Switzerland	17-9-2008	500	pos	pos	pos	pos	
ES51	*Cronobacter malonaticus*	Micr 595	DSM 18702T, Type strain		14-1-2009	445	pos	pos	pos	pos	
ES46	*Cronobacter dublinensis subsp.*	Micr 596	DSM 18706T, Type strain		14-1-2009	491	pos	pos	pos	pos	
ES49	*Cronobacter dublinensis subsp.*	Micr 597	DSM 18707T, Type strain		14-1-2009	400	pos	pos	pos	pos	
ES4	*Enterobacter sakazakii*	Micr 507	Europe	Cereals	17-9-2008	564	pos	pos	pos	pos	
ES5	*Enterobacter sakazakii*	Micr 512	Europe	Cereals	17-9-2008	727	pos	pos	pos	pos	
ES6	*Enterobacter sakazakii*	Micr 542	DSM 4485 (ATCC 29544), Type strain	Child's throat	17-9-2008	129	pos	pos	pos	pos	
ES7	*Enterobacter sakazakii*	Micr 486	Europe	Environment	17-9-2008	1027	pos	pos	pos	pos	
ES8	*Enterobacter sakazakii*	Micr 487	Europe	Environment	17-9-2008	600	pos	pos	pos	pos	
ES9	*Enterobacter sakazakii*	Micr 490	Europe	Environment	24-9-2008	773	pos	pos	pos	pos	
ES10	*Enterobacter sakazakii*	Micr 491	Europe	Environment	24-9-2008	627	pos	pos	pos	pos	
ES11	*Enterobacter sakazakii*	Micr 492	Europe	Environment	24-9-2008	718	pos	pos	pos	pos	
ES12	*Enterobacter sakazakii*	Micr 494	Europe	Environment	24-9-2008	591	pos	pos	pos	pos	
ES13	*Enterobacter sakazakii*	Micr 495	Europe	Environment	24-9-2008	709	pos	pos	pos	pos	
ES14	*Enterobacter sakazakii*	Micr 496	Europe	Environment	24-9-2008	491	pos	pos	pos	pos	
ES15	*Enterobacter sakazakii*	Micr 497	Europe	Environment	24-9-2008	882	pos	pos	pos	pos	
ES16	*Enterobacter sakazakii*	Micr 518	USA	Environment	24-9-2008	755	pos	pos	pos	pos	
ES17	*Enterobacter sakazakii*	Micr 579	Canada	Environment	24-9-2008	555	pos	pos	pos	pos	
ES18	*Enterobacter sakazakii*	Micr 573	The Netherlands	Environment: dishwash brush	24-9-2008	409	pos	pos	pos	pos	
ES19	*Enterobacter sakazakii*	Micr 563	Switzerland	Environment: sand	24-9-2008	545	pos	pos	pos	pos	
ES20	*Enterobacter sakazakii*	Micr 561	Switzerland	Environment: sponge	2-10-2008	122	pos	pos	pos	pos	
ES21	*Enterobacter sakazakii*	Micr 489	Europe	Follow-up formula	2-10-2008	108	pos	pos	pos	pos	
ES22	*Enterobacter sakazakii*	Micr 508	Europe	Follow-up formula	2-10-2008	195	pos	pos	pos	pos	
ES23	*Enterobacter sakazakii*	Micr 510	Europe	Follow-up formula	2-10-2008	132	pos	pos	pos	pos	
ES24	*Enterobacter sakazakii*	Micr 514	Europe	Follow-up formula	2-10-2008	573	pos	pos	pos	pos	
ES25	*Enterobacter sakazakii*	Micr 576	Switzerland	Fruit powder	2-10-2008	436	pos	pos	pos	pos	
ES26	*Enterobacter sakazakii*	Micr 520	Europe	Human	2-10-2008	718	pos	pos	pos	pos	
ES28	*Enterobacter sakazakii*	Micr 488	Europe	Infant formula	2-10-2008	455	pos	pos	pos	pos	
ES29	*Enterobacter sakazakii*	Micr 509	Europe	Infant formula	2-10-2008	391	pos	pos	pos	pos	
ES30	*Enterobacter sakazakii*	Micr 511	Europe	Infant formula	2-10-2008	491	pos	pos	pos	pos	
ES31	*Enterobacter sakazakii*	Micr 572	The Netherlands	Infant formula	9-10-2008	300	pos	pos	pos	pos	
ES32	*Enterobacter sakazakii*	Micr 374	France	Infant formula	9-10-2008	582	pos	pos	pos	pos	
ES33	*Enterobacter sakazakii*	Micr 581	New Zealand	Infant formula	9-10-2008	609	pos	pos	pos	pos	
ES34	*Enterobacter sakazakii*	Micr 515	Europe	Milk powder	9-10-2008	773	pos	pos	pos	pos	
ES35	*Enterobacter sakazakii*	Micr 568	Australia	Milk powder	9-10-2008	445	pos	pos	pos	pos	
ES36	*Enterobacter sakazakii*	Micr 569	Canada	Milk powder	9-10-2008	139	pos	pos	pos	pos	
ES37	*Enterobacter sakazakii*	Micr 570	Russia	Milk powder	9-10-2008	555	pos	pos	pos	pos	
ES38	*Enterobacter sakazakii*	Micr 571	The Netherlands	Milk powder	9-10-2008	327	pos	pos	pos	pos	
ES39	*Enterobacter sakazakii*	Micr 574	Indonesia	Milk powder	9-10-2008	336	pos	pos	pos	pos	
ES40	*Enterobacter sakazakii*	Micr 372	Indonesia	Milk Powder	9-10-2008	200	pos	pos	pos	pos	
ES41	*Enterobacter sakazakii*	Micr 575	France	Milk powder	9-10-2008	336	pos	pos	pos	pos	
ES42	*Enterobacter sakazakii*	Micr 580	Uruguay	Milk powder	9-10-2008	445	pos	pos	pos	pos	
ES43	*Enterobacter sakazakii*	Micr 239	NCTC 8155	Milk powder	9-10-2008	591	pos	pos	pos	pos	
ES44	*Enterobacter sakazakii*	Micr 493	Europe	Milk pudding	9-10-2008	673	pos	pos	pos	pos	
ES45	*Enterobacter sakazakii*	Micr 582	USA	Rice flour	9-10-2008	418	pos	pos	pos	pos	
ES47	*Enterobacter sakazakii*	Micr 566	ATCC 51329	unknown	14-1-2009	609	pos	pos	pos	pos	
ES48	*Enterobacter sakazakii*	Micr 559	NCTC 9844	unknown	14-1-2009	518	pos	pos	pos	pos	
ES50	*Enterobacter sakazakii*	Micr 562	Switzerland	Water: fountain	2-2-2009	636	pos*	pos*	pos*	pos*	* result after re-culture of strain (first/second runs gave repetitions)

Note: *E. sakazakii* nowadays *Cronobacter* spp.

Annex I. Inclusivity and exclusivity data from the original LR 8/9/19/20 studies (RIKILT report 2010.506), continued.

Annex XIV Raw data exclusivity study

							kit A		kit B		
							EB	ES	EB	ES	
Sample nr.	Strain	Ref. nr.	Origin	Source	Date of DNA isolation	Inoculation per 90 ml BPW (cfu)	LC 2.0	LC 2.0	IQS	IQS	Remarks
NS1	*Micrococcus luteus*	Micr 70	ATCC 9341		9-2-2009	122	neg*	neg*	neg*	neg*	* result after re-culture of strain (first/second runs gave repetition)
NS2	*Acinetobacter baumannii*	Micr 532	DSM 30007, Type strain	Urine	24-9-2008	105	neg	neg	neg	neg	
NS3	*Acinetobacter calcoaceticus*	Micr 583	The Netherlands	Milk	9-2-2009	582	neg*	neg*	neg*	neg*	* result after re-culture of strain (first/second runs gave repetition)
NS4	*Aeromonas hydrophila*	Micr 376	ATCC 7966		17-9-2008	29	neg	neg	neg	neg	
NS5	*Aeromonas media*	Micr 551	DSM 4881, Type strain	Fish farm effluent	9-2-2009	44	neg*	neg*	neg*	neg*	* result after re-culture of strain (first/second runs gave repetition)
NS6	*Aeromonas sobria*	Micr 484	ATCC 43979, Type strain	Fish	14-1-2009	72	neg	neg	neg	neg	
NS7	*Alcaligenes faecalis*	Micr 533	DSM 30030, Type strain		9-10-2008	291	neg	neg	neg	neg	
NS8	*Shewanella putrefaciens*	Micr 555	DSM 50426 (ATCC 8072)	Butter	9-2-2009	*	neg	neg	neg	neg	*no growth during pre-enrichments in BPW, tested on plate pure growth
NS9	*Bacillus cereus*	Micr 380	ATCC 14579		9-2-2009	25	neg*	neg*	neg*	neg*	* result after re-culture of strain (first/second runs gave repetition)
NS10	*Bacillus subtilus*	Micr 20		Food	17-9-2008	11	neg	neg	neg	neg	
NS11	*Chromobacterium violaceum*	Micr 609	DSM 30191, Type strain	Fresh water	14-1-2009	193	neg	neg*	neg	neg	* 2 runs with repetition results, negative interpretation visually
NS12	*Enterococcus faecalis*	Micr 176	ATCC 29212	Urine	17-9-2008	500	neg	neg	neg	neg	
NS13	*Enterococcus faecalis*	Micr 529	DSM 20478, Type strain		24-9-2008	618	neg	neg	neg	neg	
NS14	*Leuconostoc mesenteroides*	Micr 64		Food	9-2-2009	3300	neg*	neg*	neg*	neg*	* result after re-culture of strain (first/second runs gave repetition)
NS15	*Geobacillus stearothermophilus*	Micr 564	DSM 5934		9-2-2009	*	neg	neg	neg	neg	*no growth during pre-enrichments in BPW, tested on plate pure growth
NS16	*Bifidobacterium breve*	AB#9		Food	14-1-2009	*	neg	neg	neg	neg	*no growth during pre-enrichments in BPW, tested on plate pure growth
NS17	*Lactobacillus plantarum*	Micr 61	ATCC 8014		17-9-2008	71	neg	neg	neg	neg	
NS18	*Listeria monocytogenes*	List 115	The Netherlands	Cheese	2-10-2008	509	neg	neg	neg	neg	
NS19	*Moraxella (Branhamella)*	Micr 557	DSM 9143, Type strain		2-10-2008	22	neg	neg	neg	neg	
NS20	*Pasteurella aerogenes*	Micr 525	DSM 10153	Swine intestine, USA	2-10-2008	16	neg	neg	neg	neg	
NS21	*Pseudomonas aeruginosa*	Micr 378	ATCC 15442	Animal room water bottle	2-10-2008	1091	neg	neg	neg	neg	
NS22	*Pseudomonas alcaligenes*	Micr 552	DSM 50342, Type strain	Swimming-pool water	9-2-2009	591	neg*	neg*	neg*	neg*	* result after re-culture of strain (first/second runs gave repetition)
NS23	*Pseudomonas fluorescens*	Micr 377		Food	2-10-2008	227	neg	neg	neg	neg	
NS24	*Pseudomonas putida*	Micr 379	ATCC 49128		2-10-2008	77	neg	neg	neg	neg	
NS25	*Saccaromyces cerevisiae*	Micr 84	ATCC 9080		14-1-2009	*	neg	neg	neg	neg	*no growth during pre-enrichments in BPW, tested on plate pure growth
NS26	*Staphylococcus aereus*	Micr 381		Ice cream	14-1-2009	140	neg	neg	neg	neg	
NS27	*Streptococcus bovis*	Micr 166	ATCC 9809		14-1-2009	58	neg	neg	neg	neg	
NS28	*Vibrio alginolyticus*	Micr 524	The Netherlands	Oysters	14-1-2009	99	neg	neg	neg	neg	
NS29	*Usteria innocua*	List 152	NCTC 11288		14-1-2009	673	neg	neg	neg	neg	
NS30	*Bifidobacterium longum*	AB#10	NCFB 2259		14-1-2009	*	neg	neg	neg	neg	*no growth during pre-enrichments in BPW, tested on plate pure growth

Annex J. Tabulated RLOD data and calculations for the evaluation of 10 gram versus 100 gram test portions

Matrix: **Probiotic infant formula (L. reuteri)** **Inoculation strain:** ***Cronobacter sakazakii*** **NCTC 11467**

		Reference method 100 gram samples					
Sample nr.	Inoculation level (cfu/sample)	VRBG	Final confirmed result EB	CSB-CCI	Final confirmed result Crono	Data summary* EB	Data summary* Crono
533.1	0	ng	-	ng	-	0/5	0/5
533.2		ng	-	ng	-		
533.3		ng	-	ng	-		
533.4		ns	-	ng	-		
533.5		ng	-	ng	-		
533.6	1,25	ng	-	ng	-	6/20	7/20
533.8		ng	-	ng	-		
533.10		ng	-	ng	-		
533.11		ng	-	ng	-		
533.12		ng	-	ng	-		
533.14		ns	-	ns	-		
533.15		ng	-	ng	-		
533.16		ng	-	ng	-		
533.17		ng	-	ng	-		
533.19		ng	-	ng	-		
533.20		ng	-	ng	-		
533.21		ng	-	ng	-		
533.24		ng	-	ng	-		
533.9		ng	-	S	+		
533.7		S	+	S	+		
533.13		S	+	S	+		
533.18		S	+	S	+		
533.22		S	+	S	+		
533.23		S	+	S	+		
533.25		S	+	S	+		
533.26	6,25	S	+	S	+	5/5	5/5
533.27		S	+	S	+		
533.28		S	+	S	+		
533.29		S	+	S	+		
533.30		S	+	S	+		

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

		Reference method 10 gram samples					
Sample nr.	Inoculation level (cfu/sample)	SRBG	Final confirmed result EB	CSB-CCI	Final confirmed result Crono	Data summary* EB	Data summary* Crono
533.31	0	ng	-	ng	-	0/5	0/5
533.32		ng	-	ng	-		
533.33		ng	-	ng	-		
533.34		ng	-	ng	-		
533.35		ng	-	ng	-		
533.36	1,25	ng	-	ng	-	6/20	6/20
533.37		ng	-	ng	-		
533.38		ng	-	ng	-		
533.39		ng	-	ng	-		
533.40		ng	-	ng	-		
533.41		ng	-	ng	-		
533.43		ng	-	ng	-		
533.45		ng	-	ng	-		
533.50		ng	-	ng	-		
533.51		ng	-	ng	-		
533.52		ng	-	ng	-		
533.53		ng	-	ng	-		
533.54		ng	-	ng	-		
533.55		ng	-	ng	-		
533.42		S	+	S	+		
533.44		S	+	S	+		
533.46		S	+	S	+		
533.47		S	+	S	+		
533.48		S	+	S	+		
533.49		S	+	S	+		
533.60	6,25	ng	-	ng	-	3/5	3/5
533.58		ng	-	S	+		
533.59		S	+	ng	-		
533.56		S	+	S	+		
533.57		S	+	S	+		

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

Annex J. Tabulated RLOD data and calculations for the evaluation of 10 gram versus 100 gram test portions, continued.

Date:	28-9-2016	**Name:**	PIF plus probiotics		
Sample size:	10	**# matrices:**	2	**# samples:**	3

Name:	Enterobacteriaceae			
Level	n_1	n_2	y_1	y_2
0	5	5	0	0
1	20	20	6	6
2	5	5	3	5

Name:	Cronobacter			
Level	n_1	n_2	y_1	y_2
0	5	5	0	0
1	20	20	6	7
2	5	5	3	5

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Enterobacteriaceae	0,626	0,241	1,626	-0,469	0,477	0,982	1,674
Cronobacter	0,559	0,218	1,432	-0,581	0,470	1,236	1,784
Combined	0,591	0,303	1,155	-0,526	0,335	1,570	1,883

Annex K. Artificial inoculation of test samples

C + S Combination nr. (Annex O)	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 subsp. 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 subsp. lausannensis*	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

Annex K. Artificial inoculation of test samples, continued

Abbreviation (Annex O)	Strain	Strain code	Origin	Source	inoculation (cfu/sample)
Crono only	*Cronobacter sakazakii*	Lenticule	NCTC 11467	Human	6
Salm only (S97)	S. Thompson	S97		Animal feed	5,6
Salm only (S99)	S. Typhimurium	S99	1,4,[5],12:i:-	Human	4,5
EB 37	*Citrobacter freundii*	Micr 037	ATCC 8090	Type/QC strain	6
EB 80	*Proteus mirabilis*	Micr 080	ATCC 29906	Type strain	<1
EB 200	*Shigella sonnei*	Micr 200		Food	<1
EB 360	*Enterobacter cloacae*	Micr 360		Environment: milk	15,8
EB 560	*Klebsiella pneumoniae*	Micr 560		Environment: sponge	4,5
EB 737	*Serratia proteomaculans*	Micr 737		Food environment	6,2
EB 768	*Enterobacter ludwigii*	Micr 768		Food environment	12,3
EB 776	*Hafnia paralvei*	Micr 776		Food environment	<1
EB 782	*Pantoea agglomerans*	Micr 782		Food environment	0,7
EB 794	*E. coli*	Micr 794		Dairy	7

NB: Materials used for artificial inoculations were enumerated on the day of actual seeding, or within one week after the seeding procedure (re-testing due to a mistake in the dilutions series).

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.

Table L.1. Raw data sensitivity study, *additional information naturally contaminated samples*

			Reference methods				
Cat-Type	Sample nr.	Item	VRBG	Final confirmed result EB	CSB-CCI	Final confirmed result Crono	Identification (API20E or ID32E)
1-c	71	Infant cereals	S	+	S/ns	-	*Buttiauxella agrestis*
1-c	76	Breakfast light wholemeal vanilla (8+ m.)	S	+	ns	-	no identification
1-c	80	Cerealmix for porridge (6+ m.)	S/S	+/+	ns/ns	-/-	*Enterobacter cloacae*
3-a	88	Lactose	S	+	ng	-	*Enterobacter cloacae*
3-b	191	Wheat starch	S	+	ng/ng	-	*Pantoea agglomerans*
3-b	192	Wheat	S	+	ns/ns	-	no identification
3-b	194	Buckwheat flour	S	+	S	+	*C. sakazakii /C. malonaticus*
3-b	196	Oatmeal	S	+	ns/ns	-	no identification
3-b	197	Wheatflour	S	+	S	+	*Cronobacter* spp.
3-b	198	Whole wheat flour	S	+	S	+	*Cronobacter* spp.
3-b	199	Biological rye flour	S	+	S	-	*E. coli*
3-b	200	Flour	S	+	S	-	*Enterobacter cloacae*
4-a	22	Sponge equipment PIF factory	S	+	ns	-	no identification
4-a	24	Sponge equipment PIF factory	S	+	S	+	*Cronobacter sakazakii*
4-c	5	Vacuum cleaner residues	S	+	ns	-	no identification
4-c	6	Vacuum cleaner residues	S	+	ns	-	no identification
4-c	730.30	Vacuum cleaner residues	S	+	S+ns	+	*Cronobacter* spp.

Annex M. Tabulated raw data RLOD study

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

Annex N. 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:	Enterobacteriaceae - 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:	EB PIF Ref method

Level of inoculum in cfu/g or cfu/ml	No. of inoculated tubes	No. of positive tubes
d	n	y
1,75	20	17
8,75	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]			$LOD_{95\%}$ [2]			statistic matrix effect
				Detection limit	conf. limit	conf. limit	Detection limit	conf. limit	conf. limit	
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	EB PIF Ref metho	0,011	0,280	0,639	0,365	1,120	2,763	1,577	4,839	0,000
Combined data		0,011	0,280	0,639	0,365	1,120	2,763	1,577	4,839	0,000

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			statistic matrix effect
				Detection limit	Lower conf.	Upper conf.	Detection limit	Lower conf.	Upper conf.	
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	EB PIF Ref metho	0,011	0,280	0,639	0,365	1,120	2,763	1,577	4,839	0,000

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

Enterobacteriaceae	Ref method		
	$LOD_{50\%}$		
	Detection limit	Lower conf. limit	Upper conf. limit
Item	$D_{0.5,i}$	$D_{0.5,i,L}$	$D_{0.5,i,U}$
Infant formula (Crono)	0,639	0,365	1,120
Infant formula (Salm)	0,365	0,208	0,640
Infant formula containing probiotics (Crono)	1,972	1,048	3,710
Infant formula containing probiotics (Salm)	0,462	0,259	0,825
Starch (Salm)	0,295	0,169	0,516
Vacuum cleaner residues (Crono)	0,840	0,411	1,714

Cronobacter	Ref method		
	$LOD_{50\%}$		
	Detection limit	Lower conf. limit	Upper conf. limit
Item	$D_{0.5,i}$	$D_{0.5,i,L}$	$D_{0.5,i,U}$
Infant formula	0,639	0,365	1,120
Infant formula containing probiotics	1,731	0,934	3,207
Starch	0,723	0,377	1,385
Vacuum cleaner residues	0,945	0,459	1,948

Enterobacteriaceae	Magn Prep Kit IV, PCR kit A		
	$LOD_{50\%}$		
	Detection limit	Lower conf. limit	Upper conf. limit
Item	$D_{0.5,i}$	$D_{0.5,i,L}$	$D_{0.5,i,U}$
Infant formula (Crono)	0,639	0,365	1,120
Infant formula (Salm)	0,365	0,208	0,640
Infant formula containing probiotics (Crono)	1,972	1,048	3,710
Infant formula containing probiotics (Salm)	0,462	0,259	0,825
Starch (Salm)	0,340	0,193	0,599
Vacuum cleaner residues (Crono)	0,945	0,459	1,948

Cronobacter	Magn Prep Kit IV, PCR kit A		
	$LOD_{50\%}$		
	Detection limit	Lower conf. limit	Upper conf. limit
Item	$D_{0.5,i}$	$D_{0.5,i,L}$	$D_{0.5,i,U}$
Infant formula	0,639	0,365	1,120
Infant formula containing probiotics	1,972	1,048	3,710
Starch	0,723	0,377	1,385
Vacuum cleaner residues	1,071	0,515	2,228

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.

Annex O. List of participants to the ILS

Laboratory	**Country**
Analytisches Institut Bostel	Germany
Biotecon	Germany
CLF	Germany
DIL	Germany
KIN	Germany
Labor L+S	Germany
LGL Bayern	Germany
Merck KGaA	Germany
MLUA	Germany
MQD	Germany
MUVA Kempten	Germany
SGS Germany	Germany
Uni Muenchen	Germany
WESSLING Laboratorien GmbH	Germany
Centre for Food Safety, Dublin	Ireland
University of Limerick	Ireland
Götene Ingredients	Sweden
Nestlé Switzerland	Switzerland
UFAG, CH	Switzerland
FrieslandCampina	The Netherlands
RIKILT **(Expert Laboratory)**	The Netherlands

Annex P. Raw data per participant to the ILS

LAB A		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of								ABI 7300						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							-	-	NA	-	-	NA	
EBES0	EBES 19	-	-							-	-	NA	-	-	NA	
EBES0	EBES 20	-	-							-	-	NA	-	-	NA	
EBES0	EBES 27	-	-							-	-	NA	-	-	NA	
EBES0	EBES 35	-	-							-	-	NA	-	-	NA	
EBES0	EBES 38	-	-							-	-	NA	-	-	NA	
EBES0	EBES 40	-	-							-	-	NA	-	-	NA	
ES1	EBES 3	-	-							-	-	NA	-	-	NA	
ES1	EBES 33	-	-							-	-	NA	-	-	NA	
ES1	EBES 8	+	+							+	+	PA	+	+	PA	
ES1	EBES 10	+	+							+	+	PA	+	+	PA	
ES1	EBES 13	+	+							+	+	PA	+	+	PA	
ES1	EBES 16	+	+							+	+	PA	+	+	PA	
ES1	EBES 34	+	+							+	+	PA	+	+	PA	
ES1	EBES 37	+	+							+	+	PA	+	+	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB B		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 480						ABI 7500						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							rep/-	-	NA	-/-	-	NA	
EBES0	EBES 19	-	-							rep/-	-	NA	-/-	-	NA	
EBES0	EBES 20	-	-							rep/-	-	NA	-/-	-	NA	
EBES0	EBES 27	-	-							rep/-	-	NA	-/-	-	NA	
EBES0	EBES 35	-	-							rep/-	-	NA	-/-	-	NA	
EBES0	EBES 38	-	-							rep/-	-	NA	-/-	-	NA	
EBES0	EBES 40	-	-							rep/-	-	NA	-/-	-	NA	
ES1	EBES 33	-	-							rep/-	-	NA	-/-	-	NA	
ES1	EBES 34	-	-							rep/-	-	NA	-/-	-	NA	
ES1	EBES 8	+	-							rep/+	+	PA	-/-	-	NA	
ES1	EBES 3	+	+							+	+	PA	+	+	PA	
ES1	EBES 10	+	+							+	+	PA	+	+	PA	
ES1	EBES 13	+	+							+	+	PA	+	+	PA	
ES1	EBES 16	+	+							+	+	PA	+	+	PA	
ES1	EBES 37	+	+							+	+	PA	+	+	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							rep/+	+	PA	rep/+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

+	Unexpected positive for EB only, but consistent for reference and alternative method.

Annex P. Raw data per participant to the ILS, continued

LAB C		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of								iQ5						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							-	-	NA	-	-	NA	
EBES0	EBES 19	-	-							-	-	NA	-	-	NA	
EBES0	EBES 20	-	-							-	-	NA	-	-	NA	
EBES0	EBES 35	-	-							-	-	NA	-	-	NA	
EBES0	EBES 38	-	-							-	-	NA	-	-	NA	
EBES0	EBES 40	-	-							-	-	NA	-	-	NA	
EBES0	EBES 27	+	+							+	+	NA	+	+	NA	
ES1	EBES 3	-	-							-	-	NA	-	-	NA	
ES1	EBES 13	-	-							-	-	NA	-	-	NA	
ES1	EBES 16	-	-							-	-	NA	-	-	NA	
ES1	EBES 37	-	-							-	-	NA	-	-	NA	
ES1	EBES 8	+	+							+	+	PA	+	+	PA	
ES1	EBES 10	+	+							+	+	PA	+	+	PA	
ES1	EBES 33	+	+							+	+	PA	+	+	PA	
ES1	EBES 34	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	-	-							-	-	PA	-	-	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Results were considered to be an interchange of samples EBES 27 and EBES 28. Reference and alternative method results consistent.

Annex P. Raw data per participant to the ILS, continued

LAB D		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes LC 480						KIT B, '5 nuclease probes						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA							
EBES0	EBES 11	-	-	-	-	NA	-	-	NA							
EBES0	EBES 19	-	-	-	-	NA	-	-	NA							
EBES0	EBES 20	-	-	-	-	NA	-	-	NA							
EBES0	EBES 27	-	-	-	-	NA	-	-	NA							
EBES0	EBES 35	-	-	-	-	NA	-	-	NA							
EBES0	EBES 38	-	-	-	-	NA	-	-	NA							
EBES0	EBES 40	-	-	-	-	NA	-	-	NA							
ES1	EBES 3	-	-	-	-	NA	-	-	NA							
ES1	EBES 8	-	-	-	-	NA	-	-	NA							
ES1	EBES 10	-	-	-	-	NA	-	-	NA							
ES1	EBES 13	+	+	+	+	PA	+	+	PA							
ES1	EBES 16	+	+	+	+	PA	+	+	PA							
ES1	EBES 33	+	+	+	+	PA	+	+	PA							
ES1	EBES 34	+	+	+	+	PA	+	+	PA							
ES1	EBES 37	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	+	+	PA							

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB E		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes ABI 7900						
		Confirmed detection of		*Enterobacteriaceae*			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							-	-	NA	-	-	NA	
EBES0	EBES 19	-	-							-	-	NA	-	-	NA	
EBES0	EBES 20	+	-							-	-	ND	-	-	NA	1)
EBES0	EBES 27	-	-							-	-	NA	-	-	NA	
EBES0	EBES 35	-	-							-	-	NA	-	-	NA	
EBES0	EBES 38	-	-							-	-	NA	-	-	NA	
EBES0	EBES 40	-	-							-	-	NA	-	-	NA	
ES1	EBES 8	-	-							-	-	NA	-	-	NA	
ES1	EBES 13	-	-							-	-	NA	-	-	NA	
ES1	EBES 33	-	-							-	-	NA	-	-	NA	
ES1	EBES 34	-	-							-	-	NA	-	-	NA	
ES1	EBES 37	-	-							-	-	NA	-	-	NA	
ES1	EBES 3	+	+							+	+	PA	+	+	PA	
ES1	EBES 10	+	+							+	+	PA	+	+	PA	
ES1	EBES 16	+	+							+	+	PA	+	+	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result

1) Unexpected positive result for **EB**, inconsistancy between reference and alternative method. Strain not available anymore for further testing.

Annex P. Raw data per participant to the ILS, continued

LAB F		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes						KIT B, '5 nuclease probes iQ5						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							-	-	NA	-	-	NA	
EBES0	EBES 19	-	-							-	-	NA	-	-	NA	
EBES0	EBES 20	-	-							-	-	NA	-	-	NA	
EBES0	EBES 27	-	-							-	-	NA	-	-	NA	
EBES0	EBES 35	-	-							-	-	NA	-	-	NA	
EBES0	EBES 38	-	-							-	-	NA	-	-	NA	
EBES0	EBES 40	-	-							-	-	NA	-	-	NA	
ES1	EBES 3	-	-							-	-	NA	-	-	NA	
ES1	EBES 8	-	-							-	-	NA	-	-	NA	
ES1	EBES 10	-	-							-	-	NA	-	-	NA	
ES1	EBES 13	-	-							-	-	NA	-	-	NA	
ES1	EBES 33	-	-							-	-	NA	-	-	NA	
ES1	EBES 16	+	+							+	+	PA	+	+	PA	
ES1	EBES 34	+	+							+	+	PA	+	+	PA	
ES1	EBES 37	+	+							+	+	PA	+	+	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB G		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes LC 2.0						KIT B, '5 nuclease probes						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA							
EBES0	EBES 11	-	-	-	-	NA	-	-	NA							
EBES0	EBES 19	-	-	rep (+)	-	NA (FP)	rep (+)	-	NA (FP)							1)
EBES0	EBES 20	-	-	rep (+)	-	NA (FP)	rep (+)	-	NA (FP)							1)
EBES0	EBES 27	-	-	-	-	NA	-	-	NA							
EBES0	EBES 35	-	-	-	-	NA	-	-	NA							
EBES0	EBES 38	-	-	-	-	NA	-	-	NA							
EBES0	EBES 40	-	-	-	-	NA	-	-	NA							
ES1	EBES 10	-	-	-	-	NA	-	-	NA							
ES1	EBES 13	-	-	-	-	NA	-	-	NA							
ES1	EBES 16	-	-	-	-	NA	-	-	NA							
ES1	EBES 37	-	-	-	-	NA	-	-	NA							
ES1	EBES 3	+	+	+	+	PA	+	+	PA							
ES1	EBES 8	+	+	+	+	PA	+	+	PA							
ES1	EBES 33	+	+	+	+	PA	+	+	PA							
ES1	EBES 34	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	+	+	PA							

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result
1)	Repetition result (manually judged as positive), but without the possibility to repeat according to the instructions

Annex P. Raw data per participant to the ILS, continued

LAB H		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes						KIT B, '5 nuclease probes Cromo4						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							-	-	NA	-	-	NA	
EBES0	EBES 19	-	-							-	-	NA	-	-	NA	
EBES0	EBES 20	-	-							-	-	NA	-	-	NA	
EBES0	EBES 27	-	-							-	-	NA	-	-	NA	
EBES0	EBES 35	-	-							-	-	NA	-	-	NA	
EBES0	EBES 38	-	-							-	-	NA	-	-	NA	
EBES0	EBES 40	-	-							-	-	NA	-	-	NA	
ES1	EBES 3	-	-							-	-	NA	-	-	NA	
ES1	EBES 8	-	-							-	-	NA	-	-	NA	
ES1	EBES 16	-	-							-	-	NA	-	-	NA	
ES1	EBES 10	+	+							+	+	PA	+	+	PA	
ES1	EBES 13	+	+							+	+	PA	+	+	PA	
ES1	EBES 33	+	+							+	+	PA	+	+	PA	
ES1	EBES 34	+	+							+	+	PA	+	+	PA	
ES1	EBES 37	+	+							+	+	PA	+	+	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB I		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 480						LC 480						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 11	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 19	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 20	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 27	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 35	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 38	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 40	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 13	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 3	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 8	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 10	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 16	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 33	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 34	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 37	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 5	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 7	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 15	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 18	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 21	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 24	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 28	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 32	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB J		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0												
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA							
EBES0	EBES 11	-	-	-	-	NA	-	-	NA							
EBES0	EBES 19	-	-	-/-	-	NA	rep/rep	-	NA							
EBES0	EBES 27	-	-	-	-	NA	-	-	NA							
EBES0	EBES 35	-	-	-	-	NA	-	-	NA							
EBES0	EBES 38	-	-	-	-	NA	rep/rep	-	NA							
EBES0	EBES 40	-	-	-	-	NA	-	-	NA							
EBES0	EBES 20	+	-	+/+	+	PA	rep/rep	-	NA							1)
ES1	EBES 3	-	-	-	-	NA	-	-	NA							
ES1	EBES 33	-	-	-	-	NA	-	-	NA							
ES1	EBES 34	-	+	-	-	NA	-	-	ND							2)
ES1	EBES 8	+	+	+	+	PA	+	+	PA							
ES1	EBES 10	+	+	+	+	PA	+	+	PA							
ES1	EBES 13	+	+	+	+	PA	+	+	PA							
ES1	EBES 16	+	+	+	+	PA	+	+	PA							
ES1	EBES 37	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	-	-	ND							

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result

1) Unexpected positive for EB only, but consistent for reference and alternative method.

2) Might have been false-positive growth or ES-growth-only due to the very low contamination level. Strain not available anymore for further testing.

Annex P. Raw data per participant to the ILS, continued

LAB K		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0						LC 480						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 11	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 19	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 20	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 27	-	-	rep/+	+	PD	-/+	+	PD	-	-	NA	-	-	NA	1)
EBES0	EBES 35	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 38	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 40	-	-	-/+	+	PD	rep/-	-	NA	-	-	NA	-	-	NA	2)
ES1	EBES 10	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 16	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 3	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 8	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 13	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 33	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 34	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 37	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 5	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 7	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 15	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 18	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 21	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 24	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 28	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 32	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result

1) Positive-deviating EB + ES results, maybe due to the repetition testing.
Growth reported on plates from the second BPW culture at repetition testing.

2) Positive-deviating EB result, maybe due to the repetition testing.
Growth reported on plates from the second BPW culture at repetition testing.

Annex P. Raw data per participant to the ILS, continued

LAB L		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes						KIT B, '5 nuclease probes Mx 3005p						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-							-	-	NA	-	-	NA	
EBES0	EBES 11	-	-							-	-	NA	-	-	NA	
EBES0	EBES 19	-	-							-	-	NA	-	-	NA	
EBES0	EBES 20	-	-							-	-	NA	-	-	NA	
EBES0	EBES 27	-	-							-	-	NA	-	-	NA	
EBES0	EBES 35	-	-							-	-	NA	-	-	NA	
EBES0	EBES 38	-	-							-	-	NA	-	-	NA	
EBES0	EBES 40	-	-							-	-	NA	-	-	NA	
ES1	EBES 16	-	-							-	-	NA	-	-	NA	
ES1	EBES 33	-	-							-	-	NA	-	-	NA	
ES1	EBES 3	+	+							+	+	PA	+	+	PA	
ES1	EBES 8	+	+							+	+	PA	+	+	PA	
ES1	EBES 10	+	+							+	+	PA	+	+	PA	
ES1	EBES 13	+	+							+	+	PA	+	+	PA	
ES1	EBES 34	+	+							+	+	PA	+	+	PA	
ES1	EBES 37	+	+							+	+	PA	+	+	PA	
ES2	EBES 5	+	+							+	+	PA	+	+	PA	
ES2	EBES 7	+	+							+	+	PA	+	+	PA	
ES2	EBES 15	+	+							+	+	PA	+	+	PA	
ES2	EBES 18	+	+							+	+	PA	+	+	PA	
ES2	EBES 21	+	+							+	+	PA	+	+	PA	
ES2	EBES 24	+	+							+	+	PA	+	+	PA	
ES2	EBES 28	+	+							+	+	PA	+	+	PA	
ES2	EBES 32	+	+							+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB M		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes						KIT B, '5 nuclease probes						
				LC 480/LC 2.0						LC 480						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 11	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 19	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 20	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 27	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 35	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 38	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 40	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 3	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 8	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 13	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 34	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 37	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 10	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 16	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 33	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 5	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 7	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 15	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 18	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 21	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 24	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 28	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 32	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB N		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes LC 2.0						KIT B, '5 nuclease probes						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA							
EBES0	EBES 11	-	-	-	-	NA	-	-	NA							
EBES0	EBES 19	-	-	-	-	NA	-	-	NA							
EBES0	EBES 20	-	-	-	-	NA	-	-	NA							
EBES0	EBES 27	-	-	-	-	NA	-	-	NA							
EBES0	EBES 35	-	-	-	-	NA	-	-	NA							
EBES0	EBES 38	-	-	-	-	NA	-	-	NA							
EBES0	EBES 40	-	-	-	-	NA	-	-	NA							
ES1	EBES 3	+	+	+	+	PA	+	+	PA							
ES1	EBES 8	+	+	+	+	PA	+	+	PA							
ES1	EBES 10	+	+	+	+	PA	+	+	PA							
ES1	EBES 13	+	+	+	+	PA	+	+	PA							
ES1	EBES 16	+	+	+	+	PA	+	+	PA							
ES1	EBES 33	+	+	+	+	PA	+	+	PA							
ES1	EBES 34	+	+	+	+	PA	+	+	PA							
ES1	EBES 37	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	+	+	PA							

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB O		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0												
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	rep/rep	-	NA	rep/-	-	NA							
EBES0	EBES 11	-	-	rep/rep	-	NA	rep/-	-	NA							
EBES0	EBES 19	-	-	+	-	NA (FP)	+	-	NA (FP)							
EBES0	EBES 20	-	-	rep/+	-	NA (FP)	rep/rep	-	NA							
EBES0	EBES 27	-	-	-	-	NA	-	-	NA							
EBES0	EBES 35	-	-	-	-	NA	-	-	NA							
EBES0	EBES 38	-	-	-	-	NA	-	-	NA							
EBES0	EBES 40	-	-	-	-	NA	-	-	NA							
ES1	EBES 34	-	-	-/-	-	NA	rep/-	-	NA							
ES1	EBES 37	-	-	-/-	-	NA	rep/-	-	NA							
ES1	EBES 3	+	+	+	+	PA	+	+	PA							
ES1	EBES 8	+	+	+	+	PA	+	+	PA							
ES1	EBES 10	+	+	+	+	PA	+	+	PA							
ES1	EBES 13	+	+	+/+	+	PA	rep/+	+	PA							
ES1	EBES 16	+	+	+	+	PA	+	+	PA							
ES1	EBES 33	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	+	+	PA							

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result

Annex P. Raw data per participant to the ILS, continued

LAB P		Reference methods results		Alternative methods results												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0												
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA							
EBES0	EBES 11	-	-	-/-	-	NA	rep/-	-	NA							
EBES0	EBES 19	-	-	rep/+	-	NA (FP)	rep/-	-	NA							
EBES0	EBES 20	-	-	rep/-	-	NA	rep/-	-	NA							
EBES0	EBES 27	-	-	rep/+	-	NA (FP)	rep/-	-	NA							
EBES0	EBES 35	-	-	rep/+	-	NA (FP)	rep/+	-	NA (FP)							
EBES0	EBES 38	-	-	-	-	NA	-	-	NA							
EBES0	EBES 40	-	-	rep/-	-	NA	rep/-	-	NA							
ES1	EBES 16	-	-	-	-	NA	-	-	NA							
ES1	EBES 3	-	-	-/-	-	NA	rep/-	-	NA							
ES1	EBES 10	-	-	rep/-	-	NA	rep/-	-	NA							
ES1	EBES 8	+	+	+	+	PA	+	+	PA							
ES1	EBES 13	+	+	+	+	PA	+	+	PA							
ES1	EBES 33	+	+	+	+	PA	+	+	PA							
ES1	EBES 34	+	+	+	+	PA	+	+	PA							
ES1	EBES 37	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	+	+	PA							

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result

Annex P. Raw data per participant to the ILS, continued

LAB Q		Reference methods results		Alternative methods results												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0						Mx 3005P						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 11	-	-	rep/-	-	NA	-/-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 19	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 20	-	-	-	-	NA	rep/rep	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 27	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 35	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 38	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 40	-	-	rep/rep	-	NA	-/-	-	NA	+	-	NA (FP)	-	-	NA	
ES1	EBES 3	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 8	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 16	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 34	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 37	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 10	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 13	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 33	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 5	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 7	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 15	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 18	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 21	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 24	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 28	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 32	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result

Annex P. Raw data per participant to the ILS, continued

LAB R*		Reference methods results		Alternative methods results												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0						Rotorgene 3000						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	+	-		-	-		+	-		-	-		
EBES0	EBES 20	-	-	+	-		+	-		-	-		-	-		
EBES0	EBES 27	-	-	+	-		+	-		+	-		+	-		
EBES0	EBES 35	-	-	+	-		+	-		+	-		+	-		
EBES0	EBES 38	-	-	+	-		+	-		+	-		+	-		
EBES0	EBES 40	-	-	+	-		+	-		+	-		-	-		
EBES0	EBES 11	+	+	+	+		+	+		+	+		+	+		
EBES0	EBES 19	+	-	+	+		+	-		+	+		+	-		
ES1	EBES 3	-	-	+	-		+	-		+	-		+	-		
ES1	EBES 33	-	-	+	-		+	-		+	-		+	-		
ES1	EBES 37	-	-	+	-		+	-		+	-		+	-		
ES1	EBES 8	+	+	+	+		+	+		+	+		+	+		
ES1	EBES 10	+	+	+	+		+	+		+	+		+	+		
ES1	EBES 13	+	+	+	+		-	-		+	+		-	-		
ES1	EBES 16	+	+	+	+		+	+		+	+		+	+		
ES1	EBES 34	+	+	+	+		+	+		+	+		+	+		
ES2	EBES 32	-	-	+	-		+	-		+	-		+	-		
ES2	EBES 5	+	+	+	+		+	+		+	+		+	+		
ES2	EBES 7	+	+	+	+		+	+		+	+		+	+		
ES2	EBES 15	+	+	+	+		+	+		+	+		+	+		
ES2	EBES 18	+	-	+	+		+	-		+	+		+	-		
ES2	EBES 21	+	+	+	+		+	+		+	+		+	+		
ES2	EBES 24	+	+	+	+		+	+		+	+		+	+		
ES2	EBES 28	+	+	+	+		+	+		+	+		+	+		

*after culture-confirmation

+	Target detected
-	Target not detected
	Deviating result
R*	Lab rejected for statistical analysis

Annex P. Raw data per participant to the ILS, continued

LAB S		**Reference methods results**		**Alternative methods results**												Remarks
		Confirmed detection of		KIT A, hybridization probes LC 2.0						KIT B, '5 nuclease probes						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA							
EBES0	EBES 11	-	-	-	-	NA	-	-	NA							
EBES0	EBES 19	-	-	-	-	NA	-	-	NA							
EBES0	EBES 20	-	-	-	-	NA	-	-	NA							
EBES0	EBES 27	-	-	-	-	NA	-	-	NA							
EBES0	EBES 35	-	-	-	-	NA	-	-	NA							
EBES0	EBES 38	-	-	-	-	NA	-	-	NA							
EBES0	EBES 40	-	-	-	-	NA	-	-	NA							
ES1	EBES 3	-	-	-	-	NA	-	-	NA							
ES1	EBES 13	-	-	-	-	NA	-	-	NA							
ES1	EBES 8	+	+	+	+	PA	+	+	PA							
ES1	EBES 10	+	+	+	+	PA	+	+	PA							
ES1	EBES 16	+	+	+	+	PA	+	+	PA							
ES1	EBES 33	+	+	+	+	PA	+	+	PA							
ES1	EBES 34	+	+	+	+	PA	+	+	PA							
ES1	EBES 37	+	+	+	+	PA	+	+	PA							
ES2	EBES 5	+	+	+	+	PA	+	+	PA							
ES2	EBES 7	+	+	+	+	PA	+	+	PA							
ES2	EBES 15	+	+	+	+	PA	+	+	PA							
ES2	EBES 18	+	+	+	+	PA	+	+	PA							
ES2	EBES 21	+	+	+	+	PA	+	+	PA							
ES2	EBES 24	+	+	+	+	PA	+	+	PA							
ES2	EBES 28	+	+	+	+	PA	+	+	PA							
ES2	EBES 32	+	+	+	+	PA	+	+	PA							

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB T		**Reference methods results**		**Alternative methods results**												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0						iQ5						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 11	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 19	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 20	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 27	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 35	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 38	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 40	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 3	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 16	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 8	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 10	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 13	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 33	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 34	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 37	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 5	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 7	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 15	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 18	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 21	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 24	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 28	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 32	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Annex P. Raw data per participant to the ILS, continued

LAB EL		Reference methods results		Alternative methods results												Remarks
				KIT A, hybridization probes						KIT B, '5 nuclease probes						
		Confirmed detection of		LC 2.0						IQ5						
				Enterobacteriaceae			*Cronobacter*			*Enterobacteriaceae*			*Cronobacter*			
Contamination level	Sample code	EB	Crono	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	PCR result	Final Alt. Result*	Validation result	
EBES0	EBES 1	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 11	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 19	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 20	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 27	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 35	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 38	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
EBES0	EBES 40	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 34	-	-	-	-	NA	-	-	NA	-	-	NA	-	-	NA	
ES1	EBES 3	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 8	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 10	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 13	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 16	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 33	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES1	EBES 37	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 5	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 7	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 15	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 18	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 21	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 24	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 28	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	
ES2	EBES 32	+	+	+	+	PA	+	+	PA	+	+	PA	+	+	PA	

*after culture-confirmation

+	Target detected
-	Target not detected

Identical results for 7-9-2009 and 8-9-2009