Validation Study According to ISO 16140-2:2016

and MicroVal Technical Rules

Summary Report

Summary Report for the ISO 16140-2:2016 Validation of the Thermofisher Scientific™ SureTect™ *Salmonella* spp. Method (2022LR111) for the Detection of *Salmonella* spp. in a Broad Range of Foods, Animal Feed and Production Environmental Samples

Qualititative Method

Version 2
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All documents related to this study can be consulted upon request from **Thermo Fisher Scientific**
The technical protocol and results interpretation were realized according to the EN ISO 16140-2:2016. and MicroVal Validation Technical Rules

Validation protocols	EN ISO 16140: 2016 Microbiology of the food chain- Method Validation - *Part 1: Vocabulary* - *Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method* MicroVal Technical Rules
Reference method	EN ISO 6579-1:2017 *Microbiology of the food chain - Horizontal method for the detection. enumeration and serotyping of Salmonella species - Part 1: detection of Salmonella species* EN ISO 6579-1:2017/AMD 1:2020 *Amendment 1: Broader range of incubation temperatures. Annex D was not carried out during the method validation*
Studied method	SureTect Salmonella species
Already covered scopes	Broad range of foods Animal Feed Production environmental samples (excluding primary production samples)
Certification body	MicroVal www.microval.org

1 INTRODUCTION

The Thermo Scientific™ SureTect™ Salmonella Species PCR Assay was validated according to the ISO 16140:2003 standard in 2013. Multiple extension studies were run since this first validation study. and the renewal enabled to align with the revised ISO 16140-2:2016 standard in 2017.

A summary of the validation, renewal and extension studies is provided in **Table 1** below**.**

Table 1. Summary of SureTect Salmonella species Validation timeline scope. instruments and software

Studies and timeline[1]	Scope and claim	Instrument set up
Initial validation in 2013	Initial validation for ground beef meat	Thermo Scientific™ PikoReal Instrument with Thermo Scientific™ SureTect Software v1.2
Extension study in 2014	Extension to a broad range of food claim. as well as pet food	
Extension study in 2016	Extension for the 7500 Fast PCR instrument and for production environment samples	Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with Applied Biosystems™ RapidFinder™ Express Software v2.0 or later
Extension study in 2017	Extension for seafood and vegetables according to the ISO 16140-2:2016	
Renewal study in 2017	Renewal for a broad range of foods for the PikoReal thermocylcer	PikoReal Instrument with Thermo Scientific SureTect Software v1.2
Renewal study in 2018	Renewal for the use of the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	Applied Biosystems 7500 Fast Real-Time PCR Instrument with RapidFinder Express Software v2.0 or later
Extension study in 2018	Extension for the use of the QS5 PCR instrument	Applied Biosystems QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v1.0 or later
Extension study in 2018	Extension for the use of the SimpliAmp during lysis step	Applied Biosystems™ SimpliAmp™ Thermal Cycler
Extension study in 2019	Extension for Milk powder. Infant formula and Infant cereals with and without probiotics including ingredients. and for 375g sample size	Applied Biosystems 7500 Fast Real-Time PCR Instrument with RapidFinder Express Software v2.0 or later version Applied Biosystems QuantStudio 5 Real-Time PCR Instrument with Thermo Scientific RapidFinder Analysis Software v1.0 or later version
Extension in 2020	Extension of software and Assay file versions	Applied Biosystems QuantStudio 5 Real-Time PCR Instrument with Thermo Scientific RapidFinder Analysis Software v1.0 or later version Applied Biosystems 7500 Fast Real-Time PCR Instrument with RapidFinder Express Software v2.0 or later version Applied Biosystems QuantStudio 5
Extension in 2020	Extension for Cocoa and Chocolate products. and alignement of the non-selective temperature with the new ISO 6579-1:2017 rules (move from 37°C ± 1°C to 34-38°C incubation range)	
Extension in 2023	Extension for a broad range of foods and animal feed with use of pre-warmed (41.5 or 37±1°C) BPW ISO as the primary enrichment media, in addition to alternative sample sizes	Applied Biosystems QuantStudio 5 Real-Time PCR Instrument with Thermo Scientific RapidFinder Analysis Software v1.0 or later version Applied Biosystems 7500 Fast Real-Time PCR Instrument with RapidFinder Express Software v2.0 or later version Applied Biosystems QuantStudio 5

[1] Studies run at ADRIA (FR) from 2013 to 2019 and at Q-Laboratories (USA) since 2020.

2 METHODS PROTOCOLS

2.1 Alternative Method

2.1.1 Principle

The SureTect Salmonella species PCR Assay is based on gene amplification by real time polymerase chain reaction (PCR). The assay is designed to detect *Salmonella* species through the amplification of target DNA sequences which are specific to *Salmonella* species. This is accomplished through dye-labeled probes and an internal positive control (IPC). With the use of Solaris PCR technology amplification of the target sequence and IPC allows the end user to obtain results and determine if the PCR reaction has occurred in real time.
The SureTect Salmonella species PCR Assay can be used with the following platforms: Applied Biosystems™ QuantStudio™ 5 Instrument with RapidFinder™ Analysis Software v1.0 or later with Assay file Salmonella spp_SureTect_QS5 version 2.1 or later, and Applied Biosystems™ 7500 Fast Instrument with RapidFinder™ Express Software v2.0 or later with Assay file Salmonella Species SureTect 2.0 or later. Data analysis is automated and predefined on both platforms with no changes made by the end user. Results are reported as depicted below.

QuantStudio™ 5 Instrument with RapidFinder™ Analysis Software v1.0 or later:

Result icon	Result
	Positive result
	Negative result
	Result warning

7500 Fast Instrument with RapidFinder™ Express Software v2.0 or later:

Result icon[1]	Result
	Positive result
	Negative result
	Result warning

Note: QuantStudio™ 5 is noted QS5 in the rest of the document.

2.1.1 Protocol

The protocol for the alternative method is provided in the flow diagram in **Appendix 1**. Different enrichment protocols are available depending on the tested categories or the PCR instruments used. This can be found in **Table 2a** and **2b** below**.**

The multiple enrichment protocols allow the end-user to have the flexibility to decide which protocol to use based on their unique needs and preferences.

Table 2a. Enrichment Protocols Available for the PikoReal PCR Instrument

Category or type		Enrichment step	Study
1	**Meat products including poultry meats**	BPW for 20 - 28 h at 34-38°C	Initial validation study (2013)
2	**Dairy products**	BPW + 12mg/l Novobiocin for 20-28 h at 34.38°C and ONE Broth-*Salmonella* + 12 mg/l Novobiocin for 20 - 28 h at 34-38°C	Extension study (2016)
3	**Seafood and vegetables**	BPW for 20 - 28 h at 34-38°C	Initial validation study (2013)
4	**Egg products**	BPW for 20 - 28 h at 34-38°C	Initial validation study (2013)
5	**Raw beef meats with and without aromatics**	Pre-warmed BPW for 8 - 24 h at 41.5°C ± 1°C	Initial validation study (2013)
6	**Infant formula**	BPW for 16 - 24 h at 34-38°C	Renewal study
7	**Pet food**	ONE Broth-*Salmonella* + ONE Broth-*Salmonella* supplement for 20 - 28 h at 34-38°C	Initial validation study (2013)

Table 2b. Enrichment Protocols Available for the 7500 Fast and QS5 PCR Instruments

Category or type		Enrichment step	Study
1	**Meat products including poultry meats**	BPW + 12 mg/l Novobiocin for 20 - 28 h at 34-38°C	Extension study (2016)
2	**Dairy products**	BPW + 12 mg/l Novobiocin for 20 - 28 h at 34-38°C ONE Broth-*Salmonella* + 12 mg/l Novobiocin for 20 - 28 h at 34-38°C	Extension study (2016)
3	**Infant formula**	BPW for 16 - 24 h at 34-38°C	Extension study (2016)
4	**Vegetables**	BPW + 12 mg/L Novobiocin for 20 - 28 h at 34-38°C	Extension study (2017)
5	**Seafood**	BPW + 12 mg/L Novobiocin for 20 - 28 h at 34-38°C	Extension study (2017)
6	**Raw beef meats with and without aromatics**	Pre-warmed BPW for 9 - 24 h at 41.5°C ± 1°C	Extension study (2016)
7	**Environmental samples**	BPW for 20 - 28 h at 34-38°C	Extension study (2016)
8	**Milk powder. Infant formula and infant cereals with and without probiotics (Up 375g) including ingredients**	BPW + vancomycin (6 mg/L) for 18 - 26 h at 34-38°C	Extension study (2019)
9	**Cocoa and chocolate products (Up to 375 g)**	Pre-warmed BPW for 22-30 h at 34-38°C Pre-warmed UHT milk or reconstituted non-fat dried milk (NFDM) according to the ISO 6887-4:2017 standard for 20-28 h at 34-38°C	Extension Study (2020)
10	**Pet Food (375 g)**	1:6 ratio of pre-warmed (37±1°C) BPW ISO for 20-28 hours at 34-38°C	Extension Study (2023)
11	**Meat Products (375 g)**	1:5 ratio of pre-warmed (41.5±1°C) BPW ISO for 8-24 hours at 41.5±1°C	
12	**Meat Products (25 g)**	1:10 ratio of pre-warmed (41.5±1°C) BPW ISO for 8-24 hours at 41.5±1°C	
13	**Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g)**	1:6 ratio of pre-warmed (37±1°C) BPW ISO (**suppemented with 6 mg/L novobiocin for probiotic formula only)** for 18-26 hours at 34-38°C	
14	**Fresh and Processed Vegetables and Fruit (375 g)**	1:10 ratio of pre-warmed (41.5±1°C) BPW ISO for 10-24 hours at 41.5±1°C	
15	**Animal Feed (150 g)**	10 ratio BPW ISO with novobiocin at 12 mg/L for 20-28 hours at 34-38°C	
16	**Pet Food (375 g)**	1:10 ratio prewarmed (37±1°C) BPW ISO for 20-28 hours at 34-38°C	
Alternative confirmation		A direct streak of a 10 µL aliquot from the primary enrichment followed by prescribed confirmation tests without purification	

The outline of the method is presented below:

1. Enrichment following conditions listed in **Table 2a** and **2b**
2. Lysis step using 10 µL of enrichment
   - Note: For chocolates and cocoa products, enriched samples are diluted 1-in-5 in Buffer Peptone Water (BPW) prior to PCR. A 10 µL aliquot is used for lysis and DNA extraction.

3. A 20 µL aliquot of sample lysate is used for PCR.

4. Confirmation of positive results

   - Alternative Confirmation:
     Confirmation is performed from isolated characteristic colonies from the direct streak (10 µL) of the enriched sample onto XLD or Brilliance Salmonella agars* and performing:
     - Option 1: Oxoid latex test agglutination
     - Option 2: Microbact GNB 24E biochemical galleries on isolated colonies from XLD or Brilliance Salmonella agar.
     - Option 3: Any tests described in the ISO method.

     Troubleshooting: For samples with high background microflora. a sub-culture in RVS (0.1 mL primary enrichment broth in 10 mL RVS) might be required before streaking on one of the two possible selective agars.

It is possible to store the enrichment broth at 2 to 8°C for 72h. before running the lysis step of the SureTect Salmonella species protocol.

Lysates can be stored at 2 to 8°C up to 24 hours, including anytime stored at 4°C in the thermal cycler.

2.2 Restrictions

There are no restrictions.

2.3 Reference Method

The ISO standard method ISO 6579-1:2017/AMD 1: 2020 Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella* was used as the reference method. A detailed description of the method is given in **Appendix 2**.

2.4 Study design

The study design per category is listed in **Table 3**.

Table 3. Study design per category

PCR instrument	Category or type		Study design
PikoReal PCR Instrument	1	Meat products including poultry meats	Paired
	2	Dairy products	Unpaired
	3	Seafood and vegetables	Paired
	4	Egg products	Paired
	5	Raw beef meats with and without aromatics	Unpaired
	6	Infant formula	Paired
	7	Pet food	Unpaired
7500 Fast and QS5 PCR Instruments	1	Meat products	Unpaired
	2	Dairy products	Unpaired
	3	Infant formula	Paired
	4	Vegetables	Unpaired
	5	Seafood	Unpaired
	6	Raw beef meats with and without aromatics	Unpaired
	7	Environmental samples	Paired
	8	Milk powder. Infant formula and infant cereals with and without probiotics (375g) including ingredients	Unpaired
	9	Cocoa and chocolate products enriched in UHT milk or NFDM	Paired
		Cocoa and chocolate products enriched in BPW	Unpaired
	10	Pet Food (375 g)	Unpaired
	11	Meat Products (375 g)	Unpaired
	12	Meat Products (25 g)	Unpaired
	13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g)	Unpaired
	14	Fresh and Processed Vegetables and Fruit (375 g)	Unpaired
	15	Animal Feed (150 g)	Unpaired
	16	Pet Food (375 g)	Paired

3 METHOD COMPARISON STUDIES: INITIAL RENEWAL AND EXTENSION STUDIES

The method comparison study is performed by the expert laboratory in order to compare the alternative method to the reference method. The method comparison study is comprised of three parts which are tested: sensitivity study, relative limit of detection (RLOD), and inclusivity and exclusivity study.

The study was carried out on a variety of samples and strains representative of food products.

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 Number and nature of samples

Several protocols were used for (i) milk and dairy products, (ii) raw beef meats, and (iii) cocoa and chocolate products. All the possible protocol combinations noted from A to H were used for data interpretation (See **Table 4**).

Table 4. Protocol combinations used in the data interpretation

	PikoReal	7500 Fast and QS5 [1]
A	-Milk and Dairy products enriched in BPW + Novobiocin -Raw beef meats with 8 h enrichment time -All the other categories	-Milk and Dairy products enriched in BPW + Novobiocin -Raw beef meats with 9 h enrichment time -Cocoa and Chocolate products enriched in UHT milk or NFDM -All other categories from previous extensions -All 2023 extension categories minimum enrichment time
B	-Milk and Dairy products enriched ONE Broth-*Salmonella* + Novobiocin -Raw beef meats with 8 h enrichment time -All the other categories	-Dairy products ONE Broth-*Salmonella* + Novobiocin -Raw beef meats with 9 h enrichment time -Cocoa and Chocolate products enriched in UHT milk or NFDM -All other categories from previous extensions -All 2023 extension categories minimum enrichment time
C	-Milk and Dairy products enriched in BPW + Novobiocin -Raw beef meats with 24 h enrichment time -All the other categories	-Dairy products enriched in BPW + Novobiocin -Raw beef meats with 24 h enrichment time -Cocoa and Chocolate products enriched in UHT milk or NFDM -All other categories from previous extensions -All 2023 extension categories maximum enrichment time
D	-Milk and Dairy products enriched ONE Broth-*Salmonella* + Novobiocin -Raw beef meats with 24 h enrichment time -All the other categories	-Milk and Dairy products enriched ONE Broth-*Salmonella* + Novobiocin -Raw beef meats with 24 h enrichment time -Cocoa and Chocolate products enriched in UHT milk or NFDM -All other categories from previous extensions -All 2023 extension categories maximum enrichment time
E	N/A	-Milk and Dairy products enriched in BPW + Novobiocin -Raw beef meats with 9 h enrichment time -Cocoa and Chocolate products enriched in BPW -All other categories from previous extensions -All 2023 extension categories minimum enrichment time
F	N/A	-Dairy products ONE Broth-*Salmonella* + Novobiocin -Raw beef meats with 9 h enrichment time -Cocoa and Chocolate products enriched in BPW -All other categories from previous extensions -All 2023 extension categories minimum enrichment time
G	N/A	-Dairy products enriched in BPW + Novobiocin -Raw beef meats with 24 h enrichment time -Cocoa and Chocolate products enriched in BPW -All other categories from previous extensions -All 2023 extension categories maximum enrichment time
H	N/A	-Milk and Dairy products enriched ONE Broth-*Salmonella* + Novobiocin -Raw beef meats with 24 h enrichment time -Cocoa and Chocolate products enriched in BPW -All other categories from previous extensions -All 2023 extension categories maximum enrichment time

[1] For the remainder of the report "*All products*" includes all previously validated products including Dairy BPW+Novo or Diary OBS+Novo, Raw beef 9 h or 24 h, Chocolate UHT/NFDM or BPW, and all other categories form previous extensions.

The repartition of positive and negative samples per category and type is provided in **Table 5** for the PikoReal PCR Instrument and **Table 6** for the 7500 Fast and the QS5 PCR instruments.

Table 5 - Distribution per tested category and type - PikoReal PCR Instrument

Category		Type		Positive samples	Negative samples	Total
1	Meat products BPW	a	Unprocessed	9	11	20
		b	Processed	12	11	23
		c	RTE and RTRH products	13	16	29
		Total		34	38	72
2	Milks and dairy products BPW Novo	a	Pasteurized products	11	10	21
		b	Raw products	8	12	20
		c	Ingredients and low moisture products	11	10	21
		Total		30	32	62
	Milks and dairy products OBS Novo	a	Pasteurized products	12	9	21
		b	Raw products	9	11	20
		c	Ingredients and low moisture products	11	10	21
		Total		32	30	62
3	Seafood and vegetables BPW	a	Fresh. raw. frozen products	12	12	24
		b	Heat treated products	11	15	26
		c	Composite foods	9	18	27
		Total		32	45	77
4	Egg products BPW	a	Egg powders and egg-based preparations	11	9	20
		b	Liquid egg products and mayonnaises	12	12	24
		c	Egg based products	8	14	22
		Total		31	35	66
5	Raw beef meats BPW 8h	a	Fresh meats	26	18	44
		b	Frozen meats	7	16	23
		c	Seasoned meats	11	13	24
		Total		44	47	91
	Raw beef meats BPW 24h	a	Fresh meats	26	18	44
		b	Frozen meats	8	15	23
		c	Seasoned meats	11	13	24
		Total		45	46	91
6	Infant formula	a	Infant formula without probiotics	16	15	31
		b	Infant formula with probiotics	18	15	33
		Total		34	30	64
7	Pet food OBS + supl	a	Raw materials	10	17	27
		b	Low moisture products	10	11	21
		c	High moisture products	11	10	21
		Total		31	38	69
A: All products -Dairy BPW Novo -Raw beef 8h				**236**	**265**	**501**
B: All products -Dairy OBS Novo -Raw beef 8h				**238**	**263**	**501**
C: All products -Dairy BPW Novo -Raw beef 24h				**237**	**264**	**501**
D: All products -Dairy OBS Novo -Raw beef 24h				**239**	**262**	**501**

Novo: Novobiocin, OBS: ONE Broth-Salmonella

Table 6 - Distribution per tested category and type - 7500 Fast PCR and QS5 PCR Instruments

Category		Type		7500 Fast PCR Instrument			QS5 PCR Instrument		
				Positive samples	Negative samples	Total	Positive samples	Negative samples	Total
1	Meat products	a	Raw meat products (frozen or fresh)	14	16	30	14	16	30
		b	Raw poultry (fresh or frozen)	13	10	23	13	10	23
		c	Raw delicatessen	11	9	20	11	9	20
		Total		38	35	73	38	35	73
2	Milk and dairy products (BPW novo)	a	Pasteurized products	11	10	21	11	10	21
		b	Raw products	8	12	20	8	12	20
		c	Ingredients and low moisture products	11	10	21	11	10	21
		Total		30	32	62	30	32	62
	Milk and dairy products (OBS novo)	a	Pasteurized products	11	10	21	12	9	21
		b	Raw products	9	11	20	9	11	20
		c	Ingredients and low moisture products	11	10	21	11	10	21
		Total		31	31	62	32	30	62
3	Infant formula	a	Infant formula without probiotics	16	15	31	16	15	31
		b	Infant formula with probiotics	18	15	33	18	15	33
		Total		34	30	64	34	30	64
4	Vegetables	a	Raw vegetables (fresh. frozen)	10	10	20	11	9	20
		b	Processed. under atmosphere	8	12	20	8	12	20
		c	RTE	13	11	24	13	11	24
		Total		31	33	64	32	32	64
5	Seafood products	a	Raw fishery products	9	11	20	10	10	20
		b	RTRH	10	10	20	10	10	20
		c	RTE	11	12	23	11	12	23
		Total		30	33	63	31	32	63
6	Raw beef meats 9 h	a	Fresh meats	10	10	20	10	10	20
		b	Frozen meats	11	10	21	10	11	21
		c	Seasoned meats	9	11	20	9	11	20
		Total		30	31	61	29	32	61
	Raw beef meats 24 h	a	Fresh meats	10	10	20	10	10	20
		b	Frozen meats	11	10	21	11	10	21
		c	Seasoned meats	9	11	20	9	11	20
		Total		30	31	61	30	31	61
7	Environmental samples	a	Dusts and Residues	9	11	20	9	11	20
		b	Cleaning and Process Waters	11	9	20	11	9	20
		c	Surface samples	10	19	29	10	19	29
		Total		30	39	69	30	39	69
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375g)	a	Milk powder. Infant formula and infant cereals without probiotics	11	9	20	11	9	20
		b	Infant formula and infant cereals with probiotics	13	9	22	13	9	22
		c	Ingredients (Maltodextrin. starch. whey. lactose...)	9	12	21	9	12	21
		Total		33	30	63	33	30	63
9	Cocoa and chocolate products (375g) NFDM	a	Powders	14	13	27	14	13	27
		b	Chocolates	15	12	27	15	12	27
		c	Raw Ingredients	14	13	27	14	13	27
		Total		43	38	81	43	38	81
	Cocoa and chocolate products (375g) BPW	a	Powders	17	10	27	17	10	27
		b	Chocolates	17	10	27	17	10	27
		c	Raw Ingredients	17	10	27	17	10	27
		Total		51	30	81	51	30	81
10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	16	15	31	16	15	31
		b	Wet Pet Food	15	15	30	15	15	30
		Total		31	30	61	31	30	61
11	Meat Products (375 g) (8Hour)	a	Raw	13	11	24	13	11	24
		b	Seasoned and Marinated	12	11	23	12	11	23
		c	Frozen	11	9	20	11	9	20
		Total		36	31	67	36	31	67
	Meat Products (375 g) (24Hour)	a	Raw	13	11	24	13	11	24
		b	Seasoned and Marinated	13	10	23	13	10	23

Category		Type		7500 Fast PCR Instrument			QS5 PCR Instrument		
				Positive samples	Negative samples	Total	Positive samples	Negative samples	Total
12		c	Frozen	11	9	20	11	9	20
		Total		37	30	67	37	30	67
	Meat Products (25 g) (8Hour)	a	Raw	10	10	20	10	10	20
		b	Seasoned and Marinated	10	10	20	10	10	20
		c	Frozen	10	10	20	10	10	20
		Total		30	30	60	30	30	30
	Meat Products (25 g) (24Hour)	a	Raw	10	10	20	10	10	20
		b	Seasoned and Marinated	10	10	20	10	10	20
		c	Frozen	10	10	20	10	10	20
		Total		30	30	60	30	30	30
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	12	8	20	12	8	20
		b	Powdered Infant Formula (with Probiotics)	12	8	20	12	8	20
		c	Related Products/Ingredients	11	14	25	11	14	25
		Total		35	30	65	35	30	65
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	12	8	20	12	8	20
		b	Powdered Infant Formula (with Probiotics)	12	8	20	12	8	20
		c	Related Products/Ingredients	11	14	25	11	14	25
		Total		35	30	65	35	30	65
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds and Leafy Greens	14	9	23	14	9	23
		b	Vegetables	12	11	23	12	11	23
		c	Fruit and Juices	11	12	23	11	12	23
		Total		37	32	69	37	32	69
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds and Leafy Greens	14	9	23	14	9	23
		b	Vegetables	12	11	23	12	11	23
		c	Fruit and Juices	11	12	23	11	12	23
		Total		37	32	69	37	32	69
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed	14	11	25	14	11	25
		b	Livestock Feed	10	10	20	10	10	20
		c	Ingredients of Feed Products	10	10	20	10	10	20
		Total		34	31	65	34	31	65
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	15	30	15	15	30
		b	Wet Pet Food	15	15	30	15	15	30
		Total		30	30	60	30	30	60
A: All products + All Extension Categories Minimum Enrichment Time				**532**	**515**	**1047**	**533**	**514**	**1047**
B: All products + All Extension Categories Minimum Enrichment Time				**533**	**514**	**1047**	**535**	**512**	**1047**
C: All products + All Extension Categories Maximum Enrichment Time				**533**	**514**	**1047**	**535**	**512**	**1047**
D: All products + All Extension Categories Maximum Enrichment Time				**534**	**513**	**1047**	**537**	**510**	**1047**
E: All products + All Extension Categories Minimum Enrichment Time				**540**	**507**	**1047**	**540**	**507**	**1047**
F: All products + All Extension Categories Minimum Enrichment Time				**541**	**506**	**1047**	**542**	**505**	**1047**
G: All products + All Extension Categories Maximum Enrichment Time				**541**	**506**	**1047**	**542**	**505**	**1047**
H: All products + All Extension Categories Maximum Enrichment Time				**542**	**505**	**1047**	**544**	**503**	**1047**

3.1.2 Artificial contamination of samples

Artificial contamination was conducted by spiking or seeding protocols. All inoculated samples, inoculating strains, inoculation levels, and injury evaluations are provided in **Appendix 3.**

For the categories evaluated in the extension study (2023), a total of 10 (4.3 %) samples were naturally contaminated. A total of 237 samples were artificially contaminated, using 40 different strains. In total 247 contaminated (natural and artificial) samples were evaluated. A total of 234 samples gave a positive result, with 13 artificially contaminated samples being negative with non-recoverable strains. The repartition of all contaminated samples extension (including the 13 samples which produced a negative result) is presented in **Table 7a**, and the repartition of the positive samples for the extension is presented in **Table 7b**.

Table 7a. Repartition of all contaminated samples for the extension study (2023)

Artificial Contamination (CFU)			Naturally Contaminated	Total
≤ 3	3 < x < 4	4 < x ≤ 10		
196	29	12	10	247
79.4 %	11.7 %	4.9 %	4.0 %	100.0 %

Table 7b. Repartition of positive samples for the extension study (2023)

Artificial Contamination (CFU)			Naturally Contaminated	Total
≤ 3	3 < x < 4	4 < x ≤ 10		
192	28	4	10	234
82.1 %	11.9 %	1.7 %	4.3 %	100.0 %

The repartition of the positive samples for all previously validated products is given in **Tables 7c and d.** A separate table is given for Cocoa and Chocolates Products as two types of study were run.

Table 7c. Repartition of positive samples except Cocoa and Chocolate products

PCR Instruments	Naturally contaminated	Artificially contaminated					Total
		Spiking protocol			Seeding protocol		
		≤ 5 CFU	5 < x ≤ 10 CFU	>10 CFU	≤ 3 CFU	3 < x ≤ 10 CFU	
PikoReal	36	121	30	9	39	4	239
%	15.1%	50.6%	12.6%	3.8%	16.3%	1.7%	100.0%
7500 Fast	35	24	14	0	161	23	257
%	13.6%	9.3%	5.4%	0.0%	62.6%	8.9%	100.0%
QS5	35	24	14	0	163	24	260
%	13.5%	9.2%	5.4%	0.0%	62.7%	9.2%	100.0%

For the PikoReal, the 7500 Fast and the QS5 PCR instruments; 15.1 %, 13.6 %, and 13.5 % of the samples were naturally contaminated respectively.

Table 7d. Repartition of positive samples for Cocoa and Chocolate products

Artificially contaminated samples chocolate products					
Levels of contamination		**Naturally Contaminated**	**< 3 CFU**	**3 < x < 4**	**4 < x < 10**
Paired study	**Positive Samples**	3	35	5	Not run at all
	%	7%	81.4%	11.6%	
Unpaired study	**Positive Samples**	3	41	7	Not run at all
	%	6%	80.4%	13.7%	

For cocoa and chocolates products, sample types were resourced from both the United States as well as Internationally. All the artificial inoculations were strictly below 4 CFU per test portion. A total of 7.0% of the samples were naturally contaminated for cocoa and Confectionary Samples.

3.1.3 Test Results

Results obtained per category are given in **Appendix 4.**

The results obtained for the PikoReal PCR Instrument are presented in **Table 8,** in **Table 9** for the 7500 Fast PCR instrument, and in **Table 10** for the QS5 PCR instrument.

Table 8 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method results) - PikoReal PCR Instrument

Category		PA	NA	PD	ND	PPND	PPNA	Total
1	Meat products	32	37	0	2	0	1	72
2	Milk and dairy products (BPW novo)	21	32	4	4	1	0	62
	Milk & dairy products (OBS novo)	21	30	6	5	0	0	62
3	Seafood and vegetables	32	45	0	0	0	0	77
4	Egg products	31	33	0	0	0	2	66
5	Raw beef meats 8h	38	47	3	3	0	0	91
	Raw beef meats 24h	40	46	4	1	0	0	91
6	Infant formula	32	30	0	2	0	0	64
7	Pet food	28	36	2	1	0	2	69
A: All products -Dairy BPW Novo -Raw beef 8 h		**214**	**260**	**9**	**12**	**1**	**5**	**501**
B: All products -Dairy OBS Novo -Raw beef 8 h		**214**	**258**	**11**	**13**	**0**	**5**	**501**
C: All products -Dairy BPW Novo -Raw beef 24 h		**216**	**259**	**10**	**10**	**1**	**5**	**501**
D: All products -Dairy OBS Novo -Raw beef 24 h		**216**	**257**	**12**	**11**	**0**	**5**	**501**

Table 9 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method results) - 7500 Fast Instrument

	Category	PA	NA	PD	ND	PPND	PPNA	Total
1	Meat products	26	35	7	5	0	0	73
2	Milk and dairy products (BPW novo)	21	32	4	4	1	0	62
	Milk & dairy products (OBS novo)	21	31	5	5	0	0	62
3	Infant formula	33	30	0	1	0	0	64
4	Vegetables	22	33	5	3	1	0	64
5	Seafood products	22	33	3	4	1	0	63
6	Raw beef meats - 9 h	20	31	5	5	0	0	61
	Raw beef meats - 24 h	21	31	5	4	0	0	61
7	Environmental samples	27	39	0	3	0	0	69
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	21	30	6	6	0	0	63
9	Cocoa and chocolate products – Pre-warmed NFDM	43	38	0	0	0	0	81
	Cocoa and chocolate products – Pre-warmed BPW	36	30	8	7	0	0	81
10	Pet Food (375 g) (1:6 ratio)	16	30	9	5	1	0	61
11	Meat Products (375 g) (8 Hour)	17	31	11	8	0	0	67
	Meat Products (375 g) (24 Hour)	18	30	12	7	0	0	67
12	Meat Products (25 g) (8 Hour)	9	30	10	11	0	0	60
	Meat Products (25 g) (24 Hour)	9	30	10	11	0	0	60
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	27	30	7	1	0	0	65
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	27	30	7	1	0	0	65
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	18	32	9	10	0	0	69
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	18	32	9	10	0	0	69
15	Animal Feed (150 g) (20 Hour)	26	29	3	4	1	2	65
16	Animal Feed (375 g) (1:10 ratio)	29	30	1	0	0	0	60
A: All products + All Extension Categories Minimum Enrichment Time		**377**	**513**	**80**	**70**	**5**	**2**	**1047**
B: All products + All Extension Categories Minimum Enrichment Time		**377**	**512**	**81**	**71**	**4**	**2**	**1047**
C: All products + All Extension Categories Maximum Enrichment Time		**379**	**512**	**81**	**68**	**5**	**2**	**1047**
D: All products + All Extension Categories Maximum Enrichment Time		**379**	**511**	**82**	**69**	**4**	**2**	**1047**
E: All products + All Extension Categories Minimum Enrichment Time		**370**	**505**	**88**	**77**	**5**	**2**	**1047**
F: All products + All Extension Categories Minimum Enrichment Time		**370**	**504**	**89**	**78**	**4**	**2**	**1047**
G: All products + All Extension Categories Maximum Enrichment Time		**372**	**504**	**89**	**75**	**5**	**2**	**1047**
H: All products + All Extension Categories Maximum Enrichment Time		**372**	**503**	**90**	**76**	**4**	**2**	**1047**

Table 10 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative method results) - QS5 PCR instrument

Category		PA	NA	PD	ND	PPND	PPNA	Total
1	Meat products	26	35	7	5	0	0	73
2	Milk and dairy products (BPW novo)	21	32	4	4	1	0	62
	Milk & dairy products (OBS novo)	21	29	6	3	2	1	62
3	Infant formula	33	29	0	1	0	1	64
4	Vegetables	22	31	6	3	1	1	64
5	Seafood products	22	32	4	4	1	0	63
6	Raw beef meats - 9 h	21	32	4	3	1	0	61
	Raw beef meats - 24 h	21	31	5	4	0	0	61
7	Environmental samples	28	39	0	2	0	0	69
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	20	29	6	7	0	1	63
9	Cocoa and chocolate products – Pre-warmed NFDM	42	38	0	1	0	0	81
	Cocoa and chocolate products – Pre-warmed BPW	37	31	7	6	0	0	81
10	Pet Food (375 g) (1:6 ratio)	16	30	9	5	1	0	61
11	Meat Products (375 g) (8 Hour)	17	31	11	8	0	0	67
	Meat Products (375 g) (24 Hour)	18	30	12	7	0	0	67
12	Meat Products (25 g) (8 Hour)	9	30	10	11	0	0	60
	Meat Products (25 g) (24 Hour)	9	30	10	11	0	0	60
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	27	30	7	1	0	0	65
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	27	30	7	1	0	0	65
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	18	32	9	10	0	0	69
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	18	32	9	10	0	0	69
15	Animal Feed (150 g) (20 Hour)	25	30	3	6	0	1	65
16	Pet Food (375 g) (1:10 ratio)	29	30	1	0	0	0	60
A: All products + All Extension Categories Minimum Enrichment Time		**376**	**510**	**81**	**71**	**5**	**4**	**1047**
B: All products + All Extension Categories Minimum Enrichment Time		**376**	**507**	**83**	**70**	**6**	**5**	**1047**
C: All products + All Extension Categories Maximum Enrichment Time		**377**	**508**	**83**	**71**	**4**	**4**	**1047**
D: All products + All Extension Categories Maximum Enrichment Time		**377**	**505**	**85**	**70**	**5**	**5**	**1047**
E: All products + All Extension Categories Minimum Enrichment Time		**371**	**503**	**88**	**76**	**5**	**4**	**1047**
F: All products + All Extension Categories Minimum Enrichment Time		**371**	**500**	**90**	**75**	**6**	**5**	**1047**
G: All products + All Extension Categories Maximum Enrichment Time		**372**	**501**	**90**	**76**	**4**	**4**	**1047**
H: All products + All Extension Categories Maximum Enrichment Time		**372**	**498**	**92**	**75**	**5**	**5**	**1047**

3.1.4 Calculation of relative trueness (RT). sensitivity. false positive ratio (FPR)

The calculations presented in **Table 11** below were used to calculate RT, SE, and FPR. Results were calculated considering all confirmation protocols. The results and calculations are presented in **Tables 12, 13, 14 15a,** and **15b.**

Table 11. Calculations used to determine RT, SE, and FPR of sensitivity

Sensitivity for the alternative method	$\mathrm{SE_{alt}} = \frac{(\mathrm{PA}+\mathrm{PD})}{(\mathrm{PA}+\mathrm{ND}+\mathrm{PD})} \times 100\%$
Sensitivity for the alternative method	$\mathrm{SE_{ref}} = \frac{(\mathrm{PA}+\mathrm{ND})}{(\mathrm{PA}+\mathrm{ND}+\mathrm{PD})} \times 100\%$
Relative trueness	$\mathrm{AC} = \frac{(\mathrm{PA}+\mathrm{NA})}{\mathrm{N}} \times 100\%$
False positive ration for the alternative method (FP= PPNA+PPND)	$\mathrm{FP} = \frac{(\mathrm{FP})}{\mathrm{NA}} \times 100\%$

Where ND = ND + PPND and NA = NA + PPNA

Table 12 – Calculation of the relative trueness (RT), the relative sensitivity (SE) and the false positive ratio (FPR) - PikoReal PCR Instrument

Category		Type	PA	NA	PD	ND	PPND	PPNA	SE_{alt}%	SE_{ref}%	RT%	FPR%
1	Meat products BPW	a Fresh meat (unprocessed)	9	11	0	0	0	0	100.0	100.0	100.0	0
		b Processed	11	10	0	1	0	1	91.7	100.0	95.7	10.0
		c RTE and RTRH products	12	16	0	1	0	0	92.3	100.0	96.6	0.0
		Total	32	37	0	2	0	1	94.1	100.0	97.2	2.6
2	Milks and dairy products BPW Novo	a Pasteurized products	6	10	3	2	0	0	81.8	72.7	76.2	0.0
		b Raw products	6	12	0	2	0	0	75.0	100.0	90.0	0.0
		c Ingredients and low moisture products	9	10	1	0	1	0	90.9	90.9	90.5	10.0
		Total	21	32	4	4	1	0	83.3	86.7	85.5	3.1
	Milks and dairy products OBS Novo	a Pasteurized products	7	9	4	1	0	0	91.7	66.7	76.2	0.0
		b Raw products	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		c Ingredients and low moisture products	8	10	1	2	0	0	81.8	90.9	85.7	0.0
		Total	21	30	6	5	0	0	84.4	81.3	82.3	0.0
3	Seafood and vegetables BPW	a Fresh. raw . frozen products	12	12	0	0	0	0	100.0	100.0	100.0	0.0
		b Heat treated products	11	15	0	0	0	0	100.0	100.0	100.0	0.0
		c Composite foods	9	18	0	0	0	0	100.0	100.0	100.0	0.0
		Total	32	45	0	0	0	0	100.0	100.0	100.0	0.0
4	Egg products BPW	a Egg powders and egg-based preparations	11	8	0	0	0	1	100.0	100.0	100.0	12.5
		b Liquid egg products and mayonnaises	12	12	0	0	0	0	100.0	100.0	100.0	0.0
		c Egg based products	8	13	0	0	0	1	100.0	100.0	100.0	7.7
		Total	31	33	0	0	0	2	100.0	100.0	100.0	5.7
5	Raw beef meats BPW 8h	a Fresh meats	22	18	2	2	0	0	92.3	92.3	90.9	0.0
		b Frozen meats	5	16	1	1	0	0	85.7	85.7	91.3	0.0
		c Seasoned meats	11	13	0	0	0	0	100.0	100.0	100.0	0.0
		Total	38	47	3	3	0	0	93.2	93.2	93.4	0.0
	Raw beef meats BPW 24h	a Fresh meats	23	18	2	1	0	0	96.2	92.3	93.2	0.0
		b Frozen meats	6	15	2	0	0	0	100.0	75.0	91.3	0.0
		c Seasoned meats	11	13	0	0	0	0	100.0	100.0	100.0	0.0
		Total	40	46	4	1	0	0	97.8	91.1	94.5	0.0
6	Infant formula	a Infant formula without probiotics	16	15	0	0	0	0	100.0	100.0	100.0	0.0
		b Infant formula with probiotics	16	15	0	2	0	0	88.9	100.0	93.9	0.0
		Total	32	30	0	2	0	0	94.1	100.0	96.9	0.0
7	Pet food OBS + supII	a Raw materials	8	17	1	1	0	0	90.0	90.0	92.6	0.0
		b Low moisture products	9	9	1	0	0	2	100.0	90.0	95.2	22.2
		c High moisture products	11	10	0	0	0	0	100.0	100.0	100.0	0.0
		Total	28	36	2	1	0	2	96.8	93.5	95.7	5.3
A: All products - Dairy BPW Novo - Raw beef 8h			**214**	**260**	**9**	**12**	**1**	**5**	**94.5**	**96.2**	**95.6**	**2.3**
B: All products - Dairy OBS Novo - Raw beef 8h			**214**	**258**	**11**	**13**	**0**	**5**	**94.5**	**95.4**	**95.2**	**1.9**
C: All products - Dairy BPW Novo - Raw beef 24h			**216**	**259**	**10**	**10**	**1**	**5**	**95.4**	**95.8**	**95.8**	**2.3**
D: All products - Dairy OBS Novo - Raw beef 24h			**216**	**257**	**12**	**11**	**0**	**5**	**95.4**	**95.0**	**95.4**	**1.9**

Table 13 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) - 7500 Fast PCR Instrument

Category		Type		PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %
1	Meat products	a	Raw meat products (frozen or fresh)	8	16	4	2	0	0	85.7	71.4	80.0	0.0
		b	Raw poultry (fresh or frozen)	7	10	3	3	0	0	76.9	76.9	73.9	0.0
		c	Raw delicatessen	11	9	0	0	0	0	100.0	100.0	100.0	0.0
		Total		26	35	7	5	0	0	86.8	81.6	83.6	0.0
2	Milk and dairy products BPW novo	a	Pasteurized products	6	10	3	2	0	0	81.8	72.7	76.2	0.0
		b	Raw products	6	12	0	2	0	0	75.0	100.0	90.0	0.0
		c	Ingredients and low moisture products	9	10	1	0	1	0	90.9	90.9	90.5	10.0
		Total		21	32	4	4	1	0	83.3	86.7	85.5	3.1
	Milk and dairy products OBS novo	a	Pasteurized products	7	10	3	1	0	0	90.9	72.7	81.0	0.0
		b	Raw products	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		c	Ingredients and low moisture products	8	10	1	2	0	0	81.8	90.9	85.7	0.0
		Total		21	31	5	5	0	0	83.9	83.9	83.9	0.0
3	Infant formula	a	Infant formula without probiotics	16	15	0	0	0	0	100.0	100.0	100.0	0.0
		b	Infant formula with probiotics	17	15	0	1	0	0	94.4	100.0	97.0	0.0
		Total		33	30	0	1	0	0	97.1	100.0	98.4	0.0
4	Vegetables	a	Raw vegetables (fresh. frozen)	8	10	0	2	0	0	80.0	100.0	90.0	0.0
		b	Processed. under atmosphere	6	12	2	0	0	0	100.0	75.0	90.0	0.0
		c	RTE	8	11	3	1	1	0	84.6	76.9	79.2	9.1
		Total		22	33	5	3	1	0	87.1	83.9	85.9	3.0
5	Seafood products	a	Raw fishery products	8	11	0	1	0	0	88.9	100.0	95.0	0.0
		b	RTRH	10	10	0	0	0	0	100.0	100.0	100.0	0.0
		c	RTE	4	12	3	3	1	0	63.6	72.7	69.6	8.3
		Total		22	33	3	4	1	0	83.3	90.0	87.3	3.0
6	Raw beef meats 9 h	a	Fresh meats	8	10	1	1	0	0	90.0	90.0	90.0	0.0
		b	Frozen meats	6	10	3	2	0	0	81.8	72.7	76.2	0.0
		c	Seasoned meats	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		Total		20	31	5	5	0	0	83.3	83.3	83.6	0.0
	Raw beef meats 24 h	a	Fresh meats	9	10	1	0	0	0	100.0	90.0	95.0	0.0
		b	Frozen meats	6	10	3	2	0	0	81.8	72.7	76.2	0.0
		c	Seasoned meats	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		Total		21	31	5	4	0	0	86.7	83.3	85.2	0.0
7	Environmental samples	a	Dusts and Residues	7	11	0	2	0	0	77.8	100.0	90.0	0.0
		b	Cleaning and Process Waters	11	9	0	0	0	0	100.0	100.0	100.0	0.0
		c	Surface samples	9	19	0	1	0	0	90.0	100.0	96.6	0.0
		Total		27	39	0	3	0	0	90.0	100.0	95.7	0.0
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	a	Milk powder. Infant formula and infant cereals without probiotics	9	9	1	1	0	0	90.9	90.9	90.0	0.0
		b	Infant formula and infant cereals with probiotics	8	9	3	2	0	0	84.6	76.9	77.3	0.0
		c	Ingredients (Maltodextrin. starch. whey. lactose...)	4	12	2	3	0	0	66.7	77.8	76.2	0.0
		Total		21	30	6	6	0	0	81.8	81.8	81.0	0.0
9	Cocoa and chocolate products NFDM (375 g)	a	Cocoa Powder	14	13	0	0	0	0	100.0	100.0	100.0	0.0
		b	Chocolates	15	12	0	0	0	0	100.0	100.0	100.0	0.0
		c	Raw Ingredient	14	13	0	0	0	0	100.0	100.0	100.0	0.0
		Total		43	38	0	0	0	0	100.0	100.0	100.0	0.0
	Cocoa and chocolate products BPW (375 g)	a	Cocoa Powder	11	10	3	3	0	0	82.4	82.4	77.8	0.0
		b	Chocolates	13	10	2	2	0	0	88.2	88.2	85.2	0.0
		c	Raw Ingredient	12	10	3	2	0	0	88.2	82.4	81.5	0.0
		Total		36	30	8	7	0	0	86.3	84.3	81.5	0.0
10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	8	15	5	2	1	0	81.3	68.8	74.2	6.7
		b	Wet Pet Food	8	15	4	3	0	0	80.0	73.3	76.7	0.0
		Total		16	30	9	5	1	0	80.6	71.0	75.4	3.3
11	Meat Products (375 g) (8Hour)	a	Raw	8	11	2	3	0	0	76.9	84.6	79.2	0.0
		b	Seasoned and Marinated	6	11	4	2	0	0	83.3	66.7	73.9	0.0
		c	Frozen	3	9	5	3	0	0	72.7	54.5	60.0	0.0
		Total		17	31	11	8	0	0	77.8	69.4	71.6	0.0
	Meat Products (375 g) (24Hour)	a	Raw	9	11	2	2	0	0	84.6	84.6	83.3	0.0
		b	Seasoned and Marinated	6	10	5	2	0	0	84.6	61.5	69.6	0.0
		c	Frozen	3	9	5	3	0	0	72.7	54.5	60.0	0.0
		Total		18	30	12	7	0	0	81.1	67.6	71.6	0.0
12	Meat Products (25 g) (8Hour)	a	Raw	3	10	3	4	0	0	60.0	70.0	65.0	0.0
		b	Seasoned and Marinated	2	10	4	4	0	0	60.0	60.0	60.0	0.0
		c	Frozen	4	10	3	3	0	0	70.0	70.0	70.0	0.0
		Total		9	30	10	11	0	0	63.3	66.7	65.0	0.0
	Meat Products (25 g) (24Hour)	a	Raw	3	10	3	4	0	0	60.0	70.0	65.0	0.0
		b	Seasoned and Marinated	2	10	4	4	0	0	60.0	60.0	60.0	0.0
		c	Frozen	4	10	3	3	0	0	70.0	70.0	70.0	0.0
		Total		9	30	10	11	0	0	63.3	66.7	65.0	0.0
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		b	Powdered Infant Formula (with Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		c	Related Products/Ingredients	9	14	1	1	0	0	90.9	90.9	92.0	0.0
		Total		27	30	7	1	0	0	97.1	80.0	87.7	0.0
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		b	Powdered Infant Formula (with Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		c	Related Products/Ingredients	9	14	1	1	0	0	90.9	90.9	92.0	0.0
		Total		27	30	7	1	0	0	97.1	80.0	87.7	0.0

Category		Type		PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds and Leafy Greens	9	9	3	2	0	0	85.7	78.6	78.3	0.0
		b	Vegetables	4	11	4	4	0	0	66.7	66.7	65.2	0.0
		c	Fruit and Juices	5	12	2	4	0	0	63.6	81.8	73.9	0.0
		Total		18	32	9	10	0	0	73.0	75.7	72.5	0.0
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds and Leafy Greens	9	9	3	2	0	0	85.7	78.6	78.3	0.0
		b	Vegetables	4	11	4	4	0	0	66.7	66.7	65.2	0.0
		c	Fruit and Juices	5	12	2	4	0	0	63.6	81.8	73.9	0.0
		Total		18	32	9	10	0	0	73.0	75.7	72.5	0.0
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed	12	10	0	2	0	1	85.7	100.0	95.8	9.1
		b	Livestock Feed	4	9	3	2	1	1	70.0	70.0	73.7	20.0
		c	Ingredients of Feed Products	10	10	0	0	0	0	100.0	100.0	100.0	0.0
		Total		26	29	3	4	1	2	85.3	91.2	90.5	9.7
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	15	0	0	0	0	100.0	100.0	100.0	0.0
		b	Wet Pet Food	14	15	1	0	0	0	100.0	93.3	96.7	0.0
		Total		29	30	1	0	0	0	100.0	96.7	98.3	0.0
A: All products + All Extension Categories Minimum Enrichment Time				**377**	**513**	**80**	**70**	**5**	**2**	**85.9**	**85.0**	**85.4**	**1.4**
B: All products + All Extension Categories Minimum Enrichment Time				**377**	**512**	**81**	**71**	**4**	**2**	**85.9**	**84.8**	**85.3**	**1.2**
C: All products + All Extension Categories Maximum Enrichment Time				**379**	**512**	**81**	**68**	**5**	**2**	**86.3**	**84.8**	**85.5**	**1.4**
D: All products + All Extension Categories Maximum Enrichment Time				**379**	**511**	**82**	**69**	**4**	**2**	**86.3**	**84.6**	**85.4**	**1.2**
E: All products + All Extension Categories Minimum Enrichment Time				**370**	**505**	**88**	**77**	**5**	**2**	**84.8**	**83.7**	**83.9**	**1.4**
F: All products + All Extension Categories Minimum Enrichment Time				**370**	**504**	**89**	**78**	**4**	**2**	**84.8**	**83.5**	**83.8**	**1.2**
G: All products + All Extension Categories Maximum Enrichment Time				**372**	**504**	**89**	**75**	**5**	**2**	**85.2**	**83.5**	**84.0**	**1.4**
H: All products + All Extension Categories Maximum Enrichment Time				**372**	**503**	**90**	**76**	**4**	**2**	**85.2**	**83.4**	**83.9**	**1.2**

Table 14 - Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR) - QS5 PCR Instrument

Category		Type		PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %
1	Meat products	a	Raw meat products (frozen or fresh)	8	16	4	2	0	0	85.7	71.4	80.0	0.0
		b	Raw poultry (fresh or frozen)	7	10	3	3	0	0	76.9	76.9	73.9	0.0
		c	Raw delicatessen	11	9	0	0	0	0	100.0	100.0	100.0	0.0
		Total		26	35	7	5	0	0	86.8	81.6	83.6	0.0
2	Milk and dairy products BPW novo	a	Pasteurized products	6	10	3	2	0	0	81.8	72.7	76.2	0.0
		b	Raw products	6	12	0	2	0	0	75.0	100.0	90.0	0.0
		c	Ingredients and low moisture products	9	10	1	0	1	0	90.9	90.9	90.5	10.0
		Total		21	32	4	4	1	0	83.3	86.7	85.5	3.1
	Milk and dairy products OBS novo	a	Pasteurized products	7	8	4	0	1	1	91.7	66.7	76.2	22.2
		b	Raw products	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		c	Ingredients and low moisture products	8	10	1	1	1	0	81.8	90.9	85.7	10.0
		Total		21	29	6	3	2	1	84.4	81.3	82.3	10.0
3	Infant formula	a	Infant formula without probiotics	16	15	0	0	0	0	100.0	100.0	100.0	0.0
		b	Infant formula with probiotics	17	14	0	1	0	1	94.4	100.0	97.0	6.7
		Total		33	29	0	1	0	1	97.1	100.0	98.4	3.3
4	Vegetables	a	Raw vegetables (fresh. frozen)	9	8	1	1	0	1	90.9	90.9	90.0	11.1
		b	Processed, under atmosphere	5	12	2	1	0	0	87.5	75.0	85.0	0.0
		c	RTE	8	11	3	1	1	0	84.6	76.9	79.2	9.1
		Total		22	31	6	3	1	1	87.5	81.3	84.4	6.3
5	Seafood products	a	Raw fishery products	8	10	1	1	0	0	90.0	90.0	90.0	0.0
		b	RTRH	10	10	0	0	0	0	100.0	100.0	100.0	0.0
		c	RTE	4	12	3	3	1	0	63.6	72.7	69.6	8.3
		Total		22	32	4	4	1	0	83.9	87.1	85.7	3.1
6	Raw beef meats 9 h	a	Fresh meats	9	10	1	0	0	0	100.0	90.0	95.0	0.0
		b	Frozen meats	6	11	2	1	1	0	80.0	80.0	81.0	9.1
		c	Seasoned meats	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		Total		21	32	4	3	1	0	86.2	86.2	86.9	3.1
	Raw beef meats 24 h	a	Fresh meats	9	10	1	0	0	0	100.0	90.0	95.0	0.0
		b	Frozen meats	6	10	3	2	0	0	81.8	72.7	76.2	0.0
		c	Seasoned meats	6	11	1	2	0	0	77.8	88.9	85.0	0.0
		Total		21	31	5	4	0	0	86.7	83.3	85.2	0.0
7	Environmental samples	a	Dusts and Residues	7	11	0	2	0	0	77.8	100.0	90.0	0.0
		b	Cleaning and Process Waters	11	9	0	0	0	0	100.0	100.0	100.0	0.0
		c	Surface samples	10	19	0	0	0	0	100.0	100.0	100.0	0.0
		Total		28	39	0	2	0	0	93.3	100.0	97.1	0.0
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	a	Milk powder, Infant formula and infant cereals without probiotics	8	9	1	2	0	0	81.8	90.9	85.0	0.0
		b	Infant formula and infant cereals with probiotics	8	9	3	2	0	0	84.6	76.9	77.3	0.0
		c	Ingredients (Maltodextrin, starch, whey, lactose...)	4	11	2	3	0	1	66.7	77.8	76.2	9.1
		Total		20	29	6	7	0	1	78.8	81.8	79.4	3.3
9	Cocoa and chocolate products NFDM (375 g)	a	Cocoa Powder	14	13	0	0	0	0	100.0	100.0	100.0	0.0
		b	Chocolates	15	12	0	0	0	0	100.0	100.0	100.0	0.0
		c	Raw Ingredient	13	13	0	1	0	0	92.9	100.0	96.3	0.0
		Total		42	38	0	1	0	0	97.7	100.0	98.8	0.0
	Cocoa and chocolate products BPW (375 g)	a	Cocoa Powder	12	10	3	2	0	0	88.2	82.4	81.5	0.0
		b	Chocolates	13	11	1	2	0	0	87.5	93.8	88.9	0.0
		c	Raw Ingredient	12	10	3	2	0	0	88.2	82.4	81.5	0.0
		Total		37	31	7	6	0	0	88.0	86.0	84.0	0.0
10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	8	15	5	2	1	0	81.3	68.8	74.2	6.7
		b	Wet Pet Food	8	15	4	3	0	0	80.0	73.3	76.7	0.0
		Total		16	30	9	5	1	0	80.6	71.0	75.4	3.3
11	Meat Products (375 g) (8Hour)	a	Raw	8	11	2	3	0	0	76.9	84.6	79.2	0.0
		b	Seasoned and Marinated	6	11	4	2	0	0	83.3	66.7	73.9	0.0
		c	Frozen	3	9	5	3	0	0	72.7	54.5	60.0	0.0
		Total		17	31	11	8	0	0	77.8	69.4	71.6	0.0
	Meat Products (375 g) (24Hour)	a	Raw	9	11	2	2	0	0	84.6	84.6	83.3	0.0
		b	Seasoned and Marinated	6	10	5	2	0	0	84.6	61.5	69.6	0.0
		c	Frozen	3	9	5	3	0	0	72.7	54.5	60.0	0.0
		Total		18	30	12	7	0	0	81.1	67.6	71.6	0.0
12	Meat Products (25 g) (8Hour)	a	Raw	3	10	3	4	0	0	60.0	70.0	65.0	0.0
		b	Seasoned and Marinated	2	10	4	4	0	0	60.0	60.0	60.0	0.0
		c	Frozen	4	10	3	3	0	0	70.0	70.0	70.0	0.0
		Total		9	30	10	11	0	0	63.3	66.7	65.0	0.0
	Meat Products (25 g) (24Hour)	a	Raw	3	10	3	4	0	0	60.0	70.0	65.0	0.0
		b	Seasoned and Marinated	2	10	4	4	0	0	60.0	60.0	60.0	0.0
		c	Frozen	4	10	3	3	0	0	70.0	70.0	70.0	0.0
		Total		9	30	10	11	0	0	63.3	66.7	65.0	0.0
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		b	Powdered Infant Formula (with Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		c	Related Products/Ingredients	9	14	1	1	0	0	90.9	90.9	92.0	0.0
		Total		27	30	7	1	0	0	97.1	80.0	87.7	0.0
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		b	Powdered Infant Formula (with Probiotics)	9	8	3	0	0	0	100.0	75.0	85.0	0.0
		c	Related Products/Ingredients	9	14	1	1	0	0	90.9	90.9	92.0	0.0
		Total		27	30	7	1	0	0	97.1	80.0	87.7	0.0

Category		Type		PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds and Leafy Greens	9	9	3	2	0	0	85.7	78.6	78.3	0.0
		b	Vegetables	4	11	4	4	0	0	66.7	66.7	65.2	0.0
		c	Fruit and Juices	5	12	2	4	0	0	63.6	81.8	73.9	0.0
		Total		18	32	9	10	0	0	73.0	75.7	72.5	0.0
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds and Leafy Greens	9	9	3	2	0	0	85.7	78.6	78.3	0.0
		b	Vegetables	4	11	4	4	0	0	66.7	66.7	65.2	0.0
		c	Fruit and Juices	5	12	2	4	0	0	63.6	81.8	73.9	0.0
		Total		18	32	9	10	0	0	73.0	75.7	72.5	0.0
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed	12	10	0	2	0	1	85.7	100.0	95.8	9.1
		b	Livestock Feed	3	10	3	4	0	0	60.0	70.0	65.0	0.0
		c	Ingredients of Feed Products	10	10	0	0	0	0	100.0	100.0	100.0	0.0
		Total		25	30	3	6	0	1	82.4	91.2	87.5	3.2
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	15	0	0	0	0	100.0	100.0	100.0	0.0
		b	Wet Pet Food	14	15	1	0	0	0	100.0	93.3	96.7	0.0
		Total		29	30	1	0	0	0	100.0	96.7	98.3	0.0
A: All products + All Extension Categories Minimum Enrichment Time				**376**	**510**	**81**	**71**	**5**	**4**	**85.7**	**84.8**	**85.3**	**1.8**
B: All products + All Extension Categories Minimum Enrichment Time				**376**	**507**	**83**	**70**	**6**	**5**	**85.8**	**84.5**	**85.2**	**2.1**
C: All products + All Extension Categories Maximum Enrichment Time				**377**	**508**	**83**	**71**	**4**	**4**	**86.0**	**84.5**	**85.2**	**1.6**
D: All products + All Extension Categories Maximum Enrichment Time				**377**	**505**	**85**	**70**	**5**	**5**	**86.0**	**84.2**	**85.1**	**2.0**
E: All products + All Extension Categories Minimum Enrichment Time				**371**	**503**	**88**	**76**	**5**	**4**	**85.0**	**83.7**	**84.2**	**1.8**
F: All products + All Extension Categories Minimum Enrichment Time				**371**	**500**	**90**	**75**	**6**	**5**	**85.1**	**83.4**	**84.1**	**2.2**
G: All products + All Extension Categories Maximum Enrichment Time				**372**	**501**	**90**	**76**	**4**	**4**	**85.2**	**83.4**	**84.1**	**1.6**
H: All products + All Extension Categories Maximum Enrichment Time				**372**	**498**	**92**	**75**	**5**	**5**	**85.3**	**83.1**	**84.0**	**2.0**

Table 15a - Summary of results including chocolate for PikoReal, 7500 Fast and QuantStudio 5

		PikoReal PCR instrument				7500 Fast PCR instrument				QS5 PCR instrument			
		A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+ND+PD)} \times 100\%$	94.5%	94.5%	95.4%	95.4%	85.9%	85.9%	86.3%	86.3%	85.7%	85.8%	86.0%	86.0%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+ND+PD)} \; x \; 100\,\%$	96.2%	95.4%	95.8%	95.0%	85.0%	84.8%	84.8%	84.6%	84.8%	84.5%	84.5%	84.2%
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\%$	95.6%	95.2%	95.8%	95.4%	85.4%	85.3%	85.5%	85.4%	85.3%	85.2%	85.2%	85.1%
False positive ratio for the alternative method*	$FPR = \frac{(FP)}{NA} \times 100\%$	2.3%	1.9%	2.3%	1.9%	1.4%	1.2%	1.4%	1.2%	1.8%	2.1%	1.6%	2.0%

ND=ND+PPND *NA=NA+PPNA* FP=PPNA+PPND

Table 15b - Summary of results including Chocolate for 7500 Fast and QuantStudio 5

		7500 Fast PCR instrument				QS5 PCR instrument			
		E	F	G	H	E	F	G	H
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA+PD)}{(PA+ND+PD)} \times 100\%$	84.8%	84.8%	85.2%	85.2%	85.0%	85.1%	85.2%	85.3%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA+ND)}{(PA+ND+PD)} \; x \; 100\,\%$	83.7%	83.5%	83.5%	83.4%	83.7%	83.4%	83.4%	83.1%
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\%$	83.9%	83.8%	84.0%	83.9%	84.2%	84.1%	84.1%	84.0%
False positive ratio for the alternative method*	$FPR = \frac{(FP)}{NA} \times 100\%$	1.4%	1.2%	1.4%	1.2%	1.8%	2.2%	1.6%	2.0%

ND=ND+PPND *NA=NA+PPNA* FP=PPNA+PPND

A: All products + All Extension Categories Minimum Enrichment Time
B: All products + All Extension Categories Minimum Enrichment Time
C: All products + All Extension Categories Maximum Enrichment Time
D: All products + All Extension Categories Maximum Enrichment Time
E: All products + All Extension Categories Minimum Enrichment Time
F: All products + All Extension Categories Minimum Enrichment Time
G: All products + All Extension Categories Maximum Enrichment Time
H: All products + All Extension Categories Maximum Enrichment Time

3.1.5 Analysis of the discordant results

The discordant results that were observed during the sensitivity evaluation of the extension study (2023) are listed below in **Table 16a** and **16b**.

Table 16a. Discordant results for the Meat Product category of the extension study (2023)

Category	Type	Item	Inoculation Level	Sample No	SureTect Salmonella - QS5		SureTect Salmonella - 7500		Alternative Confirmation							Sample No	ISO 6579-1:2017 Confirmation								Final Agreement	
					8 Hour	24 Hour	8 Hour	24 Hour	8 Hour		24 Hour		Microbact Latex		Identification[1]	Final Result		MKTTn		RVS		Poly O	Poly H	Identification	Final Result		
					Instrument Value (CT)		Instrument Value (CT)		XLD	BSA	XLD	BSA	8Hr	24Hr				XLD	HE	XLD	HE					8 Hr	24 Hr
Meat Products (375 g)	Raw	Flanken Beef Short Ribs	natural contamination	464	-	36.05	-	36.61	m[2]	m[2]	m	m	+	+	*Salmonella* spp.	Pos	515	m	m	m	m	+	+	*Salmonella* spp.	Pos	ND	PA
	Seasoned and Marinated	Roasted Garlic & Herb Pork Tenderloin (Marinated)	natural contamination	5	-	36.47	-	35.93	m[2]	at[2]	m	m	+	+	*Salmonella* spp.	Pos	15	at	at	at	at				Neg	NA	PD

[1] Identical results produced at both minimum (8 hour) and maximum (24 hour) time points; [2] Sample was confirmed through troubleshooting procedure (see IFU).

Table 16b. Discordant results for Pet Food and Animal Feed category of the extension study (2023)

Category	Type	Item	Inoculation Level	Sample No	SureTect Salmonella – QS5	SureTect Salmonella - 7500	Alternative Confirmation				Sample No	ISO 6579-1:2017 Confirmation								Final Agreement
					20 Hour	20 Hour	20 Hour		Microbact Latex	Final Result		MKTTn		RVS		Poly O	Poly H	Identification	Final Result	
					Instrument Value (CT)		XLD	BSA				XLD	HE	XLD	HE					
Pet Food (1:6 enrichment)	Dry Pet Food	Chicken and Brown Rice Puppy Kibble	1,2,1,0,1 (1,0)	109	40.13	42.15	ng	ng		Neg	114	t	t	t	t	+	+	*Salmonella* spp.	Pos	ND
Animal Feed[1]	Pet Feed	Bird Food	4,2	1854707	-	-	t	t	+	Neg	1854707	t	t	t	t	+	+	*Salmonella* spp.	Pos	ND
		Cat Food		1854763	-	37.54	ng	ng		Neg	1854763	ng	ng	ng	ng				Neg	NA/PPNA
		Beef Dog Food		1854764	39.99	-	ng	ng		Neg	1854764	ng	ng	ng	ng				Neg	PPNA/NA
	Livestock Feed	Oats	1,0	1854717	-	37.70	ng	ng		Neg	854717	ng	ng	ng	ng				Neg	NA/PPNA
		Corn Flour	1,0	1854715	-	-	t	t	+	Neg	1854715	t	t	t	t	+	+	*Salmonella* spp.	Pos	ND
		Soybean Meal	3,0	1854779	-	40.60	t	t	+	Pos	1854779	t	t	t	t	+	+	*Salmonella* spp.	Pos	ND/PPND

[1] Note that this data was generated by MicroSept, a qualified AFNOR expert lab.

The repartition of the positive and negative deviations per type of contamination from all previously validated products is provided in **Table 17** for the PikoReal.

Table 17 - PikoReal PCR instrument

Category		Negative deviations		Positive deviations	
		Naturally contaminated	Artificially contaminated	Naturally contaminated	Artificially contaminated
1	Meat products	2	0	0	0
2	Dairy products BPW + Novobiocin	0	5	0	4
	Dairy products ONE Broth-*Salmonella* + Novobiocin	0	5	0	6
3	Seafood and vegetables	0	0	0	0
4	Egg products	0	0	0	0
5	Raw beef meats	1	2	1	3
6	Infant formula	0	2	0	0
7	Pet foods	0	1	1	1
Total (BPW)		**3**	**10**	**2**	**8**
Total (ONE Broth-*Salmonella*)		**3**	**10**	**2**	**10**

The repartition per type of contamination is similar for negative and positive deviations. *Salmonella* strains were isolated in the enrichment broth for 6 samples (10, 473, 987, 5221, 1799 and 2659). The negative deviations all previously validated products are listed in:

- **Tables 18a to c** for the PikoReal PCR Instrument;
- **Tables 19a to d** for the 7500 Fast PCR Instrument;
- **Tables 20a to d** for the QS5 PCR Instrument.

Table 18a - Negative deviations - PikoReal PCR instrument – Categories with one enrichment protocol

N° Sample	Product	Artificial contaminations		ISO 6579	Protocol	SureTect *Salmonella* - PikoReal PCR instrument					
		Strain	Inoculation level CFU/sample			PCR Result	Confirmation	Final result	Agreement	Category	Type
473	Sausages	/	/	+	BPW	-	+	-	**ND**	1	b
10	Flavored poultry meat	/	/	+	BPW	-	+	-	**ND**	1	c
615	**Infant formula with probiotics 2.9. 10^6 cfu/g**	S. Dublin Ad531	2.1	+	BPW	-	-	-	**ND**	6	b
987	**Infant formula with probiotics 5.2. 10^6 cfu/g**	S. Ohio Ad1482	1.6	+	BPW	-/-/-	+	-	**ND**	6	b
5221	**Raw materials**	S. Montevideo Ad1503	1.0	+	ONE Broth + supp	-/-/-	+	-	**ND**	7	a

Table 18b - Negative deviations - PikoReal PCR instrument – Milk and Dairy Products – Two enrichment protocols

N° Sample	Product	Artificial contaminations		ISO 6579	SureTect *Salmonella* - PikoReal PCR instrument									
					BPW + Novobiocin (12mg/L)-20h at 37°C				ONE Broth Salmonella + Novobiocin (12mg/L)-20h at 37°C				Category	Type
		Strain	Inoculation level CFU/sample		PCR Result	Confirmation	Final result	Agreement	PCR Result	Confirmation	Final result	Agreement		
486	**Pasteurized milk cheese**	S. Stourbridge Ad2297	1.6	+	i/-/-*	-	-	**ND**	+(23.38)	-	-	**PA**	2	a
489	**Pasteurized cream**	S. Duisburg Ad1812	1.6	+	-	-	-	**ND**	-	-	-	**ND**	2	a
493	**Raw milk cheese**	S. Mbandaka Ad1810	2.0	+	-	-	-	**ND**	-	-	-	**ND**	2	b
2461	**Raw milk cheese**	S. Cerro Ad2150	1.6	+	-		-	**ND**	-		-	**ND**	2	b
328	**Milk powder**	S. Tenessee Ad1171	0.6	+	+(41.28)/+(41.0)/	-	-	**PPND**	-	-	-	**ND**	2	c
330	**Milk powder**	S. Cerro Ad1173	1.3	+	+(21.2)	+	+	**PA**	-	-	-	**ND**	2	c

Table 18c - Negative deviations - PikoReal PCR instrument – Raw beef meats – Two enrichment times

N° Sample	Product	Artificial contaminations		ISO 6579	SureTect *Salmonella* - PikoReal PCR instrument									
					BPW pre-warmed (8h at 41.5°C)				BPW pre-warmed (24h at 41.5°C)				Category	Type
		Strain	Inoculation level CFU/sample		PCR Result 8h	Confirmation	Final result	Agreement	PCR Result 24h	Confirmation	Final result	Agreement		
1790	Ground beef	/	/	+	-	-	-	**ND**	-	-	-	**ND**	5	a
2659	**Ground beef**	S. Newport 586	6.0	+	-	+	-	**ND**	36.27(+)	+	+	**PA**	5	a
1799	**Frozen ground beef**	S.Panama 8	2.6	+	-	+	-	**ND**	37.51(+)	+	+	**PA**	5	b

Table 19a - Negative deviations - 7500 Fast PCR Instrument – Categories with one enrichment protocol

N°Sample	Product	Artificial contaminations		Protocol	ISO 6579 Result	SureTect *Salmonella*- 7500 Fast PCR Instrument				Category	Type
		Strain	CFU/sample			PCR reslt	All confirmatory tests	Final result	Agreement		
7477	Pork meat	/	/	P2	+(S.Rissen)	-	-	-	**ND**	1	a
7458	Chicken meat	/	/	P2	+ (S.Virchow)	-	-	-	**ND**	1	b
424	Chicken meat	/	/	P2	+ (S.Typhimurium)	-	-	-	**ND**	1	b
1194	Poultry meat	/	/	P2	+ (S.Typhimurium)	-	-	-	**ND**	1	b
615	**Infant formula with probiotics 2.9. 10^6 cfu/g**	S. Dublin Ad531	2.1	P6	+	-	-	-	**ND**	3	b
6006	**Zucchini**	S.Virchow Ad2569	0.6	P2	+	-	-	-	**ND**	4	a
6694	**Fresh spinach**	S.Havana Ad931	3.2	P2	+	-/+(30.75)/+(31.19)	+	-	**ND**	4	a
6013	**Frozen cooked potatoes**	S.Virchow F276	1.0	P2	+	-	-	-	**ND**	4	c
7046	**RTE (celery with custard)**	S.Panama Ad1733	1.8	P2	+	+(41.08)	-	-	**PPND**	4	c
6470	**Fish fillets**	S.Saintpaul F31	0.6	P2	+	-	-	-	**ND**	5	a
6016	**RTE (salad surimi pineapple)**	S.Urbana Ad2334	0.6	P2	+	-	-	-	**ND**	5	c
6472	**Seafood terrin**	S.Anatum Ad1451	0.6	P2	+	-	-	-	**ND**	5	c
7038	**Salmon terrine**	S.Anatum Ad2727	1.2	P2	+	-	-	-	**ND**	5	c
7043	**RTE (salad surimi thuna)**	S.Rubislaw Ad2332	2.0	P2	+	-	-	-	**ND**	5	c
7041	**Fish terrine**	S.Rubislaw Ad2332	2.0	P2	+	+(41.27)	-	-	**PPND**	5	c
920	Dusts (pork/beef industry)	/	/	P1	+ (S. Rissen)	-/-/-	+	-	**ND**	7	a
1543	**Dusts (sausages)**	S. Typhimurium Ad1876	2.0	P1	+	-/-/-	+	-	**ND**	7	a
7728	Wipe (meat industry)	/	/	P1	+ (S.Typhimurium)	-/-/-	+	-	**ND**	7	c
342	**Infant formula without probiotics**	S.Mbandaka Ad1810	0.3	P8	+	-/-/-	+	-	**ND**	8	a
963	**Infant cereals with probiotics (5 cereals) 3.3 10^5 cfu/g**	S.Virchow Ad1721	1.6	P8	+	-	-	-	**ND**	8	b
1240	**Infant formula with probiotics 1.3 10^6 cfu/g**	S.Anatum Ad2706	3.4	P8	+	-/-/-	+	-	**ND**	8	b
300	**Whey powder**	S.Livingstone Ad2705	0.4	P8	+	-	-	-	**ND**	8	c
303	**Maltodextrin**	S.Bovismorbificans 9	1.8	P8	+	-	-	-	**ND**	8	c
570	**Wheat flour**	S.Agona Ad1725	1.2	P8	+	-	-	-	**ND**	8	c

Table 19b - Negative deviations - 7500 Fast PCR Instrument – Milk and Dairy Products – Two enrichment protocols

N° sample	Product	Artificial contaminations		ISO 6579	SureTect *Salmonella*- 7500 Fast PCR Instrument								Category	Type
					BPW + 12mg/L Novobiocin				ONE Broth *Salmonella* + 12mg/L Novobiocin					
		Strain	Inoculation level CFU/sample		PCR result	All confirmatory tests	Final result	Agreement	PCR result	Confirmation	Final result	Agreement		
486	**Pasteurized milk cheese**	S. Stourbridge Ad2297	1.6	+	-	-	-	**ND**	+(24.41)	+	+	**PA**	2	a
489	**Pasteurized cream**	S. Duisburg Ad1812	1.6	+	-	-	-	**ND**	-	-	-	**ND**	2	a
330	**Milk powder**	S.Ceroo Ad1173	1.3	+	+(21.30)	+	+	**PA**	-	-	-	**ND**	2	b
493	**Raw milk cheese**	S. Mbandaka Ad1810	2.0	+	-	-	-	**ND**	-	-	-	**ND**	2	b
2461	**Raw milk cheese**	S.Cerro Ad2150	1.6	+	-	-	-	**ND**	-	-	-	**ND**	2	b
328	**Milk powder**	S. Tenessee Ad1171	0.6	+	+(40.18)/-/+(39.65)	-(5MSRV/ 5RVS /5MKTTn)	-	**PPND**	-	-	-	**ND**	2	c

Table 19c - Negative deviations - 7500 Fast PCR Instrument – Raw beef meats – Two enrichment times

N°Sample	Product	Artificial contaminations		ISO 6579	SureTect *Salmonella*- 7500 Fast PCR Instrument								Category	Type
					BPW pre-warmed for 9h at 41.5°C (P7)				BPW pre-warmed for 24h at 41.5°C (P7)					
		Strain	Inoculation level CFU/sample		PCR result	All confirmatory tests	Final result	Agreement	PCR result	Confirmation	Final result	Agreement		
566	**Ground beef**	S. Infantis 128	1.2	+	-/+(40.55)/+(39.61)	+	-	**ND**	+(34.55)	+	+	**PA**	6	a
443	**Frozen carpaccio**	S. Dublin Ad530	1.6	+	-		-	**ND**	-		-	**ND**	6	b
444	**Frozen carpaccio**	S. Typhimurium AOOC060	2.2	+	-		-	**ND**	i/-		-	**ND**	6	b
562	**Carpaccio**	S. Newport 586	1.2	+	-		-	**ND**	-		-	**ND**	6	c
563	**Seasoned ground beef**	S. Enteritidis Ad2295	2.4	+	-		-	**ND**	-		-	**ND**	6	c

Table 19d - Negative deviations - 7500 Fast PCR Instrument – Cocoa and Chocolate Products – Two enrichment protocols

Product	Sample Number	ISO 6579	SureTect *Salmonella*		Confirmation	7500 Fast Agreement
			7500 Fast	Ct		
Cocoa Powder	364.7	Positive	-		-	ND
White Chocolate Chip Muffin Mix	364.8	Positive	-		-	ND
Cocoa Powder	364.25	Positive	-/-/-		-	ND
Dark Chocolate	364.67	Positive	-		-	ND
Dark Chocolate	364.68	Positive	-		-	ND
Raw Organic Cacao Paste/Liquor	364.47	Positive	-/-/-		-	ND
Unrefined Cocoa Butter	364.56	Positive	-/-/-		-	ND

Table 20a - Negative deviations - QS5 PCR Instrument – Categories with one enrichment protocol

N°Sample	Product	Artificial contaminations		Protocol	ISO 6579 Result	SureTect *Salmonella* - QS5 PCR Instrument				Category	Type
		Strain	Inoculation level/sample (CFU/g)			PCR result(Cq)	All confirmatory tests	Final result	Agreement		
7477	Pork meat	/	/	P2	+(S. Rissen)	-	-	-	**ND**	1	a
7458	Chicken meat	/	/	P2	+ (S. Virchow)	-	-	-	**ND**	1	b
424	Chicken meat	/	/	P2	+ (S. Typhimurium)	-	-	-	**ND**	1	b
1194	Poultry meat	/	/	P2	+ (S. Typhimurium)	-	-	-	**ND**	1	b
615	Infant formula with probiotics 2.9. 10^6 cfu/g	S. Dublin Ad531	2.1	P6	+	-	-	-	**ND**	3	b
6006	Zucchini	S. Virchow Ad2569	0.6	P2	+	-	-	-	**ND**	4	a
6696	Baby leaves	S. Kottbus 2	3.0	P2	+	-	+	-	**ND**	4	b
6013	Frozen cooked potatoes	S. Virchow F276	1.0	P2	+	-	-	-	**ND**	4	c
7046	RTE (celeri with custard)	S. Panama Ad1733	1.8	P2	+	+(41.82)	-	-	**PPND**	4	c
6470	Fish fillets	S. Saintpaul F31	0.6	P2	+	-	-	-	**ND**	5	a
6472	Seafood terrin	S. Anatum Ad1451	0.6	P2	+	-	-	-	**ND**	5	c
6016	RTE (salad surimi pineapple)	S. Urbana Ad2334	0.6	P2	+	-	-	-	**ND**	5	c
7038	Salmon terrine	S. Anatum Ad2727	1.2	P2	+	-	-	-	**ND**	5	c
7041	Fish terrine	S. Rubislaw Ad2332	2.0	P2	+	+(41.54)	-	-	**PPND**	5	c
7043	RTE (salad surimi thuna)	S. Rubislaw Ad2332	2.0	P2	+	-	-	-	**ND**	5	c
920	Dusts (pork/beef industry)	/	/	P1	+ (S. Rissen)	-	+	-	**ND**	7	a
1543	Dusts (sausages)	S. Typhimurium Ad1876	2.0	P1	+	-	+	-	**ND**	7	a
342	Infant formula without probiotics	S. Mbandaka Ad1810	0.3	P8	+	-/-/-	+	-	**ND**	8	a
958	Skimmed milk powder	S. Ohio Ad2213	0.4	P8	+	-/+(37.06)/+(38.19)	+	-	**ND**	8	a
963	Infant cereals with probiotics (5 cereals) 3.3 10^5 cfu/g	S. Virchow Ad1721	1.6	P8	+	-	-	-	**ND**	8	b
1240	**Infant formula with probiotics 1.3 10^6 cfu/g**	S. Anatum Ad2706	3.4	P8	+	-/-/-	+	-	**ND**	8	b
300	**Whey powder**	S. Livingstone Ad2705	0.4	P8	+	-	-	-	**ND**	8	c
303	**Maltodextrin**	S. Bovismorbificans 9	1.8	P8	+	-	-	-	**ND**	8	c
570	**Wheat flour**	S. Agona Ad1725	1.2	P8	+	-	-	-	**ND**	8	c

Table 20b - Negative deviations - QS5 PCR Instrument – Milk and Dairy Products – Two enrichment protocols

N°Sample	Product	Artificial contaminations		ISO 6579 Result	SureTect *Salmonella* - QS5 PCR Instrument						Category	Type
					BPW + 12mg/L Novobiocin for 20h at 37°C (P2)			ONE Broth *Salmonella* + 12mg/L Novobiocin for 20h at 37°C (P3)				
		Strain	Inoculation level/sample (CFU/g)		PCR result(Cq)	All confirmatory tests	Agreement	PCR result(Cq)	All confirmatory tests	Agreement		
486	**Pasteurized milk cheese**	S. Stourbridge Ad2297	1.6	+	-	-	**PPND**	+(22.69)	+	**PA**	2	a
489	**Pasteurized cream**	S. Duisburg Ad1812	1.6	+	-	-	**ND**	+(36.82)	-	**PPND**	2	a
493	**Raw milk cheese**	S. Mbandaka Ad1810	2.0	+	-	-	**ND**	-	-	**ND**	2	b
2461	**Raw milk cheese**	S. Cerro Ad2150	1.6	+	-	-	**ND**	-	-	**ND**	2	b
328	**Milk powder**	S. Tenessee Ad1171	0.6	+	+(38.45)	-	**PPND**	+(38.35)	-	**PPND**	2	c
330	**Milk powder**	S. Cerro Ad1173	1.3	+	+(20.69)	+	**PA**	-	-	**ND**	2	c

Table 20c - Negative deviations - QS5 PCR Instrument – Raw beef meats – Two enrichment times

N°Sample	Product	Artificial contaminations		ISO 6579 Result	SureTect *Salmonella* - QS5 PCR Instrument BPW Pre-warmed at 41.5°C for 9 h and 24 h (P7)			Category	Type
		Strain	Inoculation level/sample (CFU/g)		PCR result(Cq)	All confirmatory tests	Agreement		
443	**Frozen carpaccio**	S. Dublin Ad530	1.6	+	+(41.59)	-	**PPND**	6	b
444	**Frozen carpaccio**	S. Typhimurium AOOC060	2.2	+	-	-	**ND**	6	b
562	**Carpaccio**	S. Newport 586	1.2	+	-	-	**ND**	6	c
563	**Seasoned ground beef**	S. Enteritidis Ad2295	2.4	+	-	-	**ND**	6	c

Table 20d - Negative deviations - QS5 PCR Instrument – Cocoa and Chocolate Products – Two enrichment protocols

Enrichment according to ISO 6887-4:2017 standard – Paired data study					
Product	Sample Number	ISO 6579 Result	Alternative Method	Confirmation	QS5 Agreement
			QS5 Ct		
Unrefined Cocoa Butter	364.56	Positive	-/-/-	+	ND
Enrichment according to ISO 6887-4:2017 standard – Unpaired data study					
Cocoa Powder	364.7	Positive	-	+	ND
White Chocolate Chip Muffin Mix	364.8	Positive	-	+	ND
Dark Chocolate	364.67	Positive	-	+	ND
Dark Chocolate	364.68	Positive	-	+	ND
Raw Organic Cacao Paste/Liquor	364.47	Positive	-/-/-	+	ND
Unrefined Cocoa Butter	364.56	Positive	-/-/-	+	ND

- 7500 Fast Instrument

Among the samples in negative deviation, the presence of *Salmonella* was confirmed for 8 samples (342, 1240, 7728, 920, 1543, 6694, 486 and 566). The contamination in the enrichment broths for these samples was probably below the limit of detection of the SureTect Salmonella method.

- QS5 PCR Instrument

Among the samples in negative deviation, the presence of *Salmonella* was confirmed for 5 samples (6696, 920, 342, 958, 1240). The contamination in the enrichment broths for these samples was probably below the limit of detection of the SureTect Salmonella method.
Note that for sample 958 (skimmed milk powder) a positive result was obtained for the first PCR test using the 7500 Fast PCR instrument (+40.88/-/+39.51) while a negative result was obtained using the QS5 PCR instrument (-/+ 37.06/+ 38.19).

The positive deviations for all previously validated products are listed in:
- **Tables 21a to c** for the PikoReal PCR Instrument;
- **Tables 22a to d** for the 7500 Fast PCR Instrument;
- **Tables 23a to d** for the QS5 PCR Instrument.

Table 21a – Positive deviations - PikoReal PCR instrument – Categories with one enrichment protocol

N°Sample	Product	Artificial contaminations		SureTect *Salmonella* - PikoReal PCR Instrument			
		Strain	Inoculation level CFU/sample	PCR result	Agreement	Category	Type
5543	Raw materials (raw meat)	/	/	37.36(+)	PD	7	a
5237	Pellets for dog (cereals. chicken. rice)	S. Agona A00V038	1.5	27.4(+)	PD	7	b

Table 21b – Positive deviations - PikoReal PCR instrument – Milk and Dairy Products – Two enrichment protocols

N°Sample	Product	Artificial contaminations		SureTect *Salmonella* - PikoReal PCR Instrument					
				BPW+Novobiocin 20h at 37°C		ONE Broth Salmonella + Novobiocin 20h at 37°C		Category	Type
		Strain	Inoculation level CFU/sample	PCR result	Agreement	PCR result	Agreement		
488	Fermented milk	S. Ohio Ad2213	1.8	-	NA	+(22.14)	PD	2	a
491	Cream	S. Dublin Ad1336	0.8	+(21.32)	PD	+(20.04)	PD	2	a
492	Raw milk	S. Stourbridge Ad2297	1.6	i/i/-*	NA	+(36.26)	PD	2	a
783	Ice cream	S. Mbandaka Ad1810	2.2	+(20.29)	PD	+(20.72)	PD	2	a
781	Ice cream Vanilla	S. Stourbridge Ad2297	1.2	+(20.02)	PD	-	NA	2	a
494	Raw milk cheese	S. Ohio Ad2213	1.8	-	NA	+(31.26)	PD	2	b
327	Milk powder	S. Cerro Ad1173	1.0	+(20.85)	PD	+(20.56)	PD	2	c

Table 21c – Positive deviations - PikoReal PCR instrument – Raw beef meats – Two enrichment times

N°Sample	Product	Artificial contaminations		SureTect *Salmonella* - PikoReal PCR Instrument					
				BPW pre-warmed 8h at 41.5°C		BPW pre-warmed 24h at 41.5°C		Category	Type
		Strain	Inoculation level CFU/sample	PCR result	Agreement	PCR result	Agreement		
1794	Ground beef	/	/	35.87(+)	PD	27.4(+)	PD	5	a
2670	Ground beef	S. Dublin Ad529	3.8	41.96(+)	PD	36.35(+)	PD	5	a
1797	Frozen ground beef	S. Infantis 128	2.8	35.07(+)	PD	35.35(+)	PD	5	b
2652	Frozen ground beef	S. Newport 586	0.8	-	NA	32.97(+)	PD	5	b

Table 22a – Positive deviations – 7500 Fast instrument – Categories with one enrichment protocol

N°Sample	Product	Artificial contaminations		Protocol	ISO 6579 Result	SureTect *Salmonella*- 7500 Fast			Category	Type
		Strain	Inoculation level CFU/sample			PCR result	Confirmation	Agreement		
483	Veal meat	S. Panama 4255	1.8	P2	-	+(25.82)	+	PD	1	a
484	Beef meat	S. Give 436	3.0	P2	-	+(20.56)	+	PD	1	a
7469	Pork meat	/	/	P2	-	+(25.72)	+	PD	1	a
7472	Pork meat	/	/	P2	-	+(34.03)	+	PD	1	a
7464	Duck meat	/	/	P2	-	+(30.00)	+	PD	1	b
7465	Duck meat	/	/	P2	-	+(31.27)	+	PD	1	b
7476	Turkey meat	/	/	P2	-	+(34.42)	+	PD	1	b
6008	Mixed vegetables under modified atmosphere	S. Livingstone Ad2566	0.8	P2	-	+(32.62)	+	PD	4	b
6010	Salad under modified atmosphere	S. Virchow F276	1.0	P2	-	+(28.64)	+	PD	4	b
6476	Celery deli salad	S. Typhimurium Ad1249	0.8	P2	-	+(21.51)	+	PD	4	c
6480	Celery deli salad	S. Virchow Ad2569	1.4	P2	-	+(27.08)	+	PD	4	c
7047	RTE (macédoine)	S. Agona Ad1725	1.4	P2	-	+(21.33)	+	PD	4	c
6015	RTE (salad rice thuna)	S. Wandsworth Ad2335	1.2	P2	-	+(21.18)	+	PD	5	c
6471	Surimi	S. Saintpaul F31	0.6	P2	-	+(22.70)	+	PD	5	c
6473	Seafood terrin	S. Indiana 2	0.6	P2	-	+(22.69)	+	PD	5	c
98	Infant formula with probiotics 7.2 10^6 cfu/g	S. Anatum Ad1168	3.1	P8	-	+(18.51)	+	PD	8	b
309	Infant cereals with probiotics (5 cereals flavor) 1.8 10^6 cfu/g	S. Panama Ad1733	1.2	P8	-	+(34.15)	+	PD	8	b
965	Infant cereals with probiotics (biscuit flavor) 4.9 10^4 cfu/g	S. Oranienburg Ad1724	1.4	P8	-	+(38.34)	+	PD	8	b
301	Caseinates	S. Stourbridge Ad2297	0.4	P8	-	+(25.09)	+	PD	8	c
567	Barley flour	S. Virchow Ad1721	0.4	P8	-	+(24.76)	+	PD	8	c

Table 22b – Positive deviations – 7500 Fast PCR instrument – Milk and Dairy Products – Two enrichment protocols

N°Sample	Product	Artificial contaminations		ISO 6579 Result	SureTect *Salmonella*- 7500 Fast PCR Instrument BPW + 12mg/L Novobiocin for 20h at 37°C			SureTect *Salmonella*- 7500 Fast PCR Instrument ONE Broth Salmonella + 12mg/L novobiocin for 20h at 37°C		
		Strain	Inoculation level CFU/sample		PCR result	Confirmation	Agreement	PCR result	Confirmation	Agreement
488	Fermented milk	S. Ohio Ad2213	1.8	-	-	-	NA	+(22.07)	+	PD
491	Cream	S. Dublin Ad1336	0.8	-	+(21.07)	+	PD	+(21.47)	+	PD
781	Ice cream (vanilla)	S. Stourbridge Ad2297	1.2	-	+(22.04)	+	PD	-	-	NA
783	Ice cream	S. Mbandaka Ad1810	2.2	-	+(22.08)	+	PD	+(22.10)	+	PD
494	Raw milk cheese	S. Ohio Ad2213	1.8	-	-	-	NA	+(32.84)	+	PD
327	Milk powder	S. Cerro Ad1173	1.0	-	+(22.55)	+	PD	+(21.29)	+	PD

Table 22c – Positive deviations – 7500 Fast PCR instrument – Raw beef meats – Two enrichment times

N°Sample	Product	Artificial contaminations		ISO 6579	SureTect *Salmonella*- 7500 Fast PCR Instrument BPW pre-warmed for 9h at 41.5°C			SureTect *Salmonella*- 7500 Fast PCR Instrument BPW pre-warmed for 24h at 41.5°C		
		Strain	Inoculation level CFU/sample		PCR result	Confirmation	Agreement	PCR result	Confirmation	Agreement
862	Veal meat (blanquette)	S. Enteritidis Ad2294	3.2	-	+(32.45)	+	PD	i/+(31.29)	+	PD
440	Frozen beef trim	S. Dublin Ad530	1.6	-	+(47.60)	+	PD	+(23.75)	+	PD
448	Frozen ground beef	S. Panama 4255	2.2	-	+(34.17)	+	PD	+(22.55)	+	PD
450	Frozen beef trim	S. Typhimurium ACC0060	2.2	-	+(29.42)	+	PD	+(21.05)	+	PD
573	Seasoned ground beef	S. Newport 586	1.2	-	+(32.08)	+	PD	+(28.07)	+	PD

22cl – Positive deviations – 7500 Fast PCR instrument – Cocoa and Chocolate Products – Two enrichment protocols

Product	Sample Number	ISO 6579 Result	7500 Fast	Ct	Confirmation	7500 Fast Agreement
Cocoa Powder	364.5	Negative	+	23.92	+	PD
Cocoa Powder	364.6	Negative	+	24.19	+	PD
Cocoa Powder	364.18	Negative	+	23.78	+	PD
85% Cocoa Dark Chocolate	364.29	Negative	+	41.69	+	PD
White Chocolate Chips	364.34	Negative	+	21.25	+	PD
Cocoa Butter Refined	364.52	Negative	+	45.55	+	PD
Organic Cacao Paste	364.53	Negative	+	41.6	+	PD
Organic Raw Cacao Beans	364.55	Negative	+	44.82	+	PD

Table 23a – Positive deviations – QS5 instrument – Categories with one enrichment protocol

N°Sample	Product	Artificial contaminations		Protocol	ISO 6579 Result	SureTect *Salmonella* - QS5 PCR Instrument			Category	Type
		Strain	Inoculation level CFU/sample			PCR result (Cq)	Confirmation	Agreement		
483	Veal meat	S. Panama 4255	1.8	P2	-	+(25.73)	+	PD	1	a
484	Beef meat	S. Give 436	3.0	P2	-	+(19.87)	+	PD	1	a
7469	Pork meat	/	/	P2	-	+(27.48)	+	PD	1	a
7472	Pork meat	/	/	P2	-	+(32.09)	+	PD	1	a
7464	Duck meat	/	/	P2	-	+(31.47)	+	PD	1	b
7465	Duck meat	/	/	P2	-	+(33.44)	+	PD	1	b
7476	Turkey meat	/	/	P2	-	+(37.53)	+	PD	1	b
6693	Coriander	S. Amsterdam	2.4	P2	-	+(34.62)	+	PD	4	a
6008	Mixed vegetables under modified atmosphere	S. Livingstone Ad2566	0.8	P2	-	+(31.20)	+	PD	4	b
6010	Salad under modified atmosphere	S. Virchow F276	1.0	P2	-	+(25.08)	+	PD	4	b
6476	Celery deli salad	S. Typhimurium Ad1249	0.8	P2	-	+(20.76)	+	PD	4	c
6480	Celery deli salad	S. Virchow Ad2569	1.4	P2	-	+(24.91)	+	PD	4	c
7047	RTE (macedoine)	S. Agona Ad1725	1.4	P2	-	+(20.33)	+	PD	4	c
6015	RTE (salad rice tuna)	S. Wandsworth Ad2335	1.2	P2	-	+(18.97)	+	PD	5	c
6467	Fish fillets	S. Derby F81	1.0	P2	-	+(38.30)	+	PD	5	a
6471	Surimi	S. Saintpaul F31	0.6	P2	-	+(22.30)	+	PD	5	c
6473	Seafood terrin	S. Indiana 2	0.6	P2	-	+(20.22)	+	PD	5	c
297	Skimmed milk powder	S. Livingstone Ad2705	0.4	P8	-	+(22.47)	+	PD	8	a
98	Infant formula with probiotics 7.2 10⁶ cfu/g	S. Anatum Ad1168	3.1	P8	-	+(18.51)	+	PD	8	b
309	Infant cereals with probiotics (5 cereals flavor) 1.8 10⁶ cfu/g	S. Panama Ad1733	1.2	P8	-	+(34.15)	+	PD	8	b
965	Infant cereals with probiotics (biscuit flavor) 4.9 10⁴ cfu/g	S. Oranienburg Ad1724	1.4	P8	-	+(38.34)	+	PD	8	b
301	Caseinates	S. Stourbridge Ad2297	0.4	P8	-	+(25.09)	+	PD	8	c
567	Barley flour	S. Virchow Ad1721	0.4	P8	-	+(24.76)	+	PD	8	c

Table 23b – Positive deviations – QS5 PCR instrument – Milk and Dairy Products – Two enrichment protocols

N°sample	Product	Artificial contaminations		ISO 6579	Alternative method: SureTect *Salmonella* - QS5 PCR Instrument Category								Category	Type
					BPW+ 12mg/L novobiocin				One Broth Salmonella for 20h at 37°C (P3)					
		Strain	Inoculation level CFU/sample		PCR result (Cq)	Confirmation	Final result	Agreement	PCR result(Cq)	Confirmation	Final result	Agreement		
488	Fermented milk	S. Ohio Ad2213	2-1-1-2-3 (1.8)	-	-	-	-	NA	+(22.38)	+	+	PD	2	a
491	Cream	S. Dublin Ad1336	1-0-2-0-1 (0.8)	-	+(21.09)	+	+	PD	+(22.63)	+	+	PD	2	a
783	Ice cream	S. Mbandaka Ad1810	3-4-2-1-1 (2.2)	-	+(21.69)	+	+	PD	+(19.24)	+	+	PD	2	a
492	Raw milk	S. Stourbridge Ad2297	2-0-3-1-2 (1.6)	-	-	+	-	NA	+(41.74)	+	+	PD	2	b
494	Raw milk cheese	S. Ohio Ad2213	2-1-1-2-3 (1.8)	-	-	-	-	NA	+(32.94)	+	+	PD	2	b
327	Milk powder	S. Cerro Ad1173	1	-	+(20.64)	+	+	PD	+(18.69)	+	+	PD	2	c
781	Ice cream (vanilla)	S. Stourbridge Ad2297	2-1-1-2-0 (1.2)	-	+(20.27)	+	+	PD	-	-	-	NA	2	c

Table 23c – Positive deviations – QS5 PCR instrument – Raw beef meats – Two enrichment times

N°Sample	Product	Artificial contaminations		ISO 6579	Alternative method: SureTect *Salmonella* - QS5 PCR Instrument								Category	Type
					Pre-warmed BPW for 9h at 41.5°C (P7)				Pre-warmed BPW for 24h at 41.5°C (P7)					
		Strain	Inoculation level CFU/sample		PCR result(Cq)	Confirmation	Final result	Agreement	PCR result(Cq)	Confirmation	Final result	Agreement		
862	Veal meat (blanquette)	S. Enteritidis Ad2294	3.2	-	+(31.70	+	+	PD	i/+(31.29)	+	+	PD	6	a
440	Frozen beef trim	S. Dublin Ad530	1.6	-	-	+	-	NA	+(24.16)	+	+	PD	6	b
448	Frozen ground beef	S. Panama 4255	2.2	-	+(25.11)	+	+	PD	+(22.55)	+	+	PD	6	b
450	Frozen beef trim	S. Typhimurium ACC0060	2.2	-	+(22.62)	+	+	PD	+(21.05)	+	+	PD	6	b
573	Seasoned ground beef	S. Newport 586	1.2	-	+(27.13)	+	+	PD	+(28.07)	+	+	PD	6	c

23d – Positive deviations – QS5 PCR instrument – Cocoa and Chocolate Products – Two enrichment protocols

Product	Sample Number	ISO 6579 Result	7500 Fast	Ct	Confirmation	7500 Fast Agreement
Cocoa Powder	364.5	Negative	+	21.91	+	PD
Cocoa Powder	364.6	Negative	+	22.14	+	PD
Cocoa Powder	364.18	Negative	+	22.49	+	PD
White Chocolate Chips	364.34	Negative	+	39.25	+	PD
Cocoa Butter Refined	364.52	Negative	+	22.56	+	PD
Organic Cacao Paste	364.53	Negative	+	22.73	+	PD
Organic Raw Cacao Beans	364.55	Negative	+	39.05	+	PD

The difference between (ND – PD) for the paired and unpaired studies and the sum of (ND + PD) for the paired studies were calculated. and the compared to the Acceptability Limits (AL) defined in the ISO 16140-2:2016 standard for one category. Analysis of discordant results according to EN ISO 16140-2:2016 are presented in **Tables 24 to 26.**

Table 24 - Analyses of discordant results - PikoReal PCR Instrument

Category		Type		N+	ND	PPND	PD	Unpaired		Paired				Combined	
								(ND+PPND)-PD	AL	(ND+PPND)-PD	AL	ND+PPND+PD	AL	(ND+PPND)-PD	AL
1	Meat products BPW	a	Unprocessed	9	0	0	0			0		0		0	
		b	Processed	12	1	0	0			1	/	1	/	1	
		c	RTE and RTRH products	13	1	0	0			1		1		1	
		Total		34	2	0	0			2	3	2	6	2	
2	Milks and dairy products BPW Novo	a	Pasteurized products	11	2	0	3	-1						-1	
		b	Raw products	8	2	0	0	2	/					2	
		c	Ingredients and low moisture products	11	0	1	1	0						0	
		Total		30	4	1	4	1	3					1	
	Milks and dairy products OBS Novo	a	Pasteurized products	12	1	0	4	-3						-3	
		b	Raw products	9	2	0	1	1	/					1	
		c	Ingredients and low moisture products	11	2	0	1	1						1	
		Total		32	5	0	6	-1	3					-1	
3	Seafood and vegetables BPW	a	Fresh, raw, frozen products	12	0	0	0			0		0		0	
		b	Heat treated products	11	0	0	0			0	/	0	/	0	
		c	Composite foods	9	0	0	0			0		0		0	
		Total		32	0	0	0			0	3	0	6	0	
4	Egg products BPW	a	Egg powders	11	0	0	0			0		0		0	
		b	Liquid egg products	12	0	0	0			0	/	0	/	0	
		c	Egg based products	8	0	0	0			0		0		0	
		Total		31	0	0	0			0	3	0	6	0	
5	Raw beef meats BPW 8h	a	Fresh meats	26	2	0	2	0						0	
		b	Frozen meats	7	1	0	1	0	/					0	
		c	Seasoned meats	11	0	0	0	0						0	
		Total		44	3	0	3	0	3					0	
	Raw beef meats BPW 24h	a	Fresh meats	26	1	0	2	-1						-1	
		b	Frozen meats	8	0	0	2	-2	/					-2	
		c	Seasoned meats	11	0	0	0	0						0	
		Total		45	1	0	4	-3	3					-3	
6	Infant formula	a	Infant formula without probiotics	16	0	0	0			0	/	0	/	0	
		b	Infant formula with probiotics	18	2	0	0			2		2		2	
		Total		34	2	0	0			2	3	2	6	2	
7	Pet food OBS + supll	a	Raw materials	10	1	0	1	0						0	
		b	Low moisture products	10	0	0	1	-1	/					-1	
		c	High moisture products	11	0	0	0	0						0	
		Total		31	1	0	2	-1	3					-1	
A: All products -Dairy BPW Novo -Raw beef 8h				**236**	**12**	**1**	**9**							**4**	**7**
B: All products -Dairy OBS Novo -Raw beef 8h				**238**	**13**	**0**	**11**							**2**	
C: All products -Dairy BPW Novo -Raw beef 24h				**237**	**10**	**1**	**10**							**1**	
D: All products -Dairy OBS Novo -Raw beef 24h				**239**	**11**	**0**	**12**							**-1**	

Table 25 – Analyses of discordant results - 7500 Fast PCR instrument

Category		Type		N+	ND	PPND	PD	Unpaired (ND+PPND)-PD	Unpaired AL	Paired (ND+PPND)-PD	Paired AL	Paired ND+PPND+PD	Paired AL	Combined (ND+PPND)-PD	Combined AL
1	Meat products	a	Raw meat products (frozen or fresh)	14	2	0	4	-2						-2	
		b	Raw poultry (fresh or frozen)	13	3	0	3	0	/					0	
		c	Raw delicatessen	11	0	0	0	0						0	
		Total		38	5	0	7	-2	3					-2	
2	Milk and dairy products BPW novo	a	Pasteurized products	11	2	0	3	-1						-1	
		b	Raw products	8	2	0	0	2	/					2	
		c	Ingredients and low moisture products	11	0	1	1	0						0	
		Total		30	4	1	4	1	3					1	
	Milk and dairy products OBS novo	a	Pasteurized products	11	1	0	3	-2						-2	
		b	Raw products	9	2	0	1	1	/					1	
		c	Ingredients and low moisture products	11	2	0	1	1						1	
		Total		31	5	0	5	0	3					0	
3	Infant formula	a	Infant formula without probiotics	16	0	0	0			0	/	0	/	0	
		b	Infant formula with probiotics	18	1	0	0			1		1		1	
		Total		34	1	0	0			1	3	1	6	1	
4	Vegetables	a	Raw vegetables (fresh, frozen)	10	2	0	0	2						2	
		b	Processed, under atmosphere	8	0	0	2	-2	/					-2	
		c	RTE	13	1	1	3	-1						-1	
		Total		31	3	1	5	-1	3					-1	
5	Seafood products	a	Raw fishery products	9	1	0	0	1						1	
		b	RTRH	10	0	0	0	0	/					0	
		c	RTE	11	3	1	3	1						1	
		Total		30	4	1	3	2	3					2	
6	Raw beef meats 9 h	a	Fresh meats	10	1	0	1	0						0	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		30	5	0	5	0	3					0	
	Raw beef meats 24 h	a	Fresh meats	10	0	0	1	-1						-1	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		30	4	0	5	-1	3					-1	
7	Environmental samples	a	Dusts and Residues	9	2	0	0			2	/	2	/	2	
		b	Cleaning and Process Waters	11	0	0	0			0		0		0	
		c	Surface samples	10	1	0	0			1		1		1	
		Total		30	3	0	0			3	3	3	6	3	
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	a	Milk powder. Infant formula and infant cereals without probiotics	11	1	0	1	0						0	
		b	Infant formula and infant cereals with probiotics	13	2	0	3	-1	/					-1	
		c	Ingredients (Maltodextrin, starch, whey, lactose…)	9	3	0	2	1						1	
		Total		33	6	0	6	0	3					0	
9	Cocoa and Chocolate products NFDM (375 g)	a	Cocoa Powder	14	0	0	0			0	/	0	/	0	
		b	Chocolates	15	0	0	0			0		0		-1	
		c	Raw Ingredients	14	0	0	0			0		0		1	
		Total		43	0	0	0			0	3	0	6	0	
	Cocoa and Chocolate products BPW (375 g)	a	Cocoa Powder	17	3	0	3	0						0	
		b	Chocolates	17	2	0	2	0	/					0	
		c	Raw Ingredients	17	2	0	3	-1						-1	
		Total		51	7	0	8	-1	3					-1	
10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	15	2	1	5	-2						-2	
		b	Wet Pet Food	15	3	0	4	-1	/					-1	
		Total		30	5	1	9	-3	3					-3	

Category		Type		N+	ND	PPND	PD	Unpaired (ND+PPND)-PD	Unpaired AL	Paired (ND+PPND)-PD	Paired AL	Paired ND+PPND+PD	Paired AL	Combined (ND+PPND)-PD	Combined AL
11	Meat Products (375 g) (8Hour)	a	Raw	13	3	0	2	1						1	
		b	Seasoned & Marinated	13	2	0	4	-2	/					-2	
		c	Frozen	11	3	0	5	-2						-2	
		Total		37	8	0	11	-3	3					-3	
	Meat Products (375 g) (24Hour)	a	Raw	13	2	0	2	0						0	
		b	Seasoned & Marinated	13	2	0	5	-3	/					-3	
		c	Frozen	11	3	0	5	-2						-2	
		Total		37	7	0	12	-5	3					-5	
12	Meat Products (25 g) (8Hour)	a	Raw	10	4	0	3	1						1	
		b	Seasoned & Marinated	10	4	0	4	0	/					0	
		c	Frozen	10	3	0	3	0						0	
		Total		30	11	0	10	1	3					1	
	Meat Products (25 g) (24Hour)	a	Raw	10	4	0	3	1						1	
		b	Seasoned & Marinated	10	4	0	4	0	/					0	
		c	Frozen	10	3	0	3	0						0	
		Total		30	11	0	10	1	3					1	
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3						-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3	/					-3	
		c	Related Products/Ingredients	11	1	0	1	0						0	
		Total		35	1	0	7	-6	3					-6	
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3						-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3	/					-3	
		c	Related Products/Ingredients	11	1	0	1	0						-0	
		Total		35	1	0	7	-6	3					-6	
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1						-1	
		b	Vegetables	12	4	0	4	0	/					0	
		c	Fruit & Juices	11	4	0	2	2						2	
		Total		37	10	0	9	1	3					1	
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1						-1	
		b	Vegetables	12	4	0	4	0	/					0	
		c	Fruit & Juices	11	4	0	2	2						2	
		Total		37	10	0	9	1	3					1	
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed	14	2	0	0	2						2	
		b	Livestock Feed	10	2	1	3	0	/					0	
		c	Ingredients of Feed Products	10	0	0	0	0						0	
		Total		34	4	1	3	2	3					2	
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	0	0	0			0		0		0	
		b	Wet Pet Food	15	1	0	0			-1		1		-1	
		Total		30	1	0	0			-1	3	1	6	-1	
A: All products + All Extension Categories Minimum Enrichment Time				**532**	**70**	**5**	**80**							**-5**	
B: All products + All Extension Categories Minimum Enrichment Time				**533**	**71**	**4**	**81**							**-6**	
C: All products + All Extension Categories Maximum Enrichment Time				**533**	**68**	**5**	**81**							**-8**	
D: All products + All Extension Categories Maximum Enrichment Time				**534**	**69**	**4**	**82**							**-9**	
E: All products + All Extension Categories Minimum Enrichment Time				**540**	**77**	**5**	**88**							**-6**	**7**
F: All products + All Extension Categories Minimum Enrichment Time				**541**	**78**	**4**	**89**							**-7**	
G: All products + All Extension Categories Maximum Enrichment Time				**541**	**75**	**5**	**89**							**-9**	
H: All products + All Extension Categories Maximum Enrichment Time				**542**	**76**	**4**	**90**							**-10**	

Table 26 - Analyses of discordant results - QS5 PCR instrument

Category		Type		N+	ND	PPND	PD	Unpaired (ND+PPND)-PD	Unpaired AL	Paired (ND+PPND)-PD	Paired AL	Paired ND+PPND+PD	Paired AL	Combined (ND+PPND)-PD	Combined AL
1	Meat products	a	Raw meat products (frozen or fresh)	14	2	0	4	-2						-2	
		b	Raw poultry (fresh or frozen)	13	3	0	3	0	/					0	
		c	Raw delicatessen	11	0	0	0	0						0	
		Total		38	5	0	7	-2	3					-2	
2	Milk and dairy products BPW novo	a	Pasteurized products	11	2	0	3	-1						-1	
		b	Raw products	8	2	0	0	2	/					2	
		c	Ingredients and low moisture products	11	0	1	1	0						0	
		Total		30	4	1	4	1	3					1	
	Milk and dairy products OBS novo	a	Pasteurized products	12	0	1	4	-3						-3	
		b	Raw products	9	2	0	1	1	/					1	
		c	Ingredients and low moisture products	11	1	1	1	1						1	
		Total		32	3	2	5	-1	3					-1	
3	Infant formula	a	Infant formula without probiotics	16	0	0	0			0	/	0	/	0	
		b	Infant formula with probiotics	18	1	0	0			1		1		1	
		Total		34	1	0	0			1	3	1	6	1	
4	Vegetables	a	Raw vegetables (fresh. frozen)	11	1	0	1	0						0	
		b	Processed, under atmosphere	8	1	0	2	-1	/					-1	
		c	RTE	13	1	1	3	-1						-1	
		Total		32	3	1	6	-2	3					-2	
5	Seafood products	a	Raw fishery products	10	1	0	1	0						0	
		b	RTRH	10	0	0	0	0	/					0	
		c	RTE	11	3	1	3	1						1	
		Total		31	4	1	3	1	3					1	
6	Raw beef meats 9 h	a	Fresh meats	10	0	0	1	-1						-1	
		b	Frozen meats	10	1	1	2	0	/					0	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		29	3	1	4	0	3					0	
	Raw beef meats 24 h	a	Fresh meats	10	0	0	1	-1						-1	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		30	4	0	5	-1	3					-1	
7	Environmental samples	a	Dusts and Residues	9	2	0	0			2	/	2	/	2	
		b	Cleaning and Process Waters	11	0	0	0			0		0		0	
		c	Surface samples	10	0	0	0			0		0		0	
		Total		30	2	0	0			2	3	2	6	2	
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	a	Milk powder. Infant formula and infant cereals without probiotics	11	2	0	1	1						1	
		b	Infant formula and infant cereals with probiotics	13	2	0	3	-1	/					-1	
		c	Ingredients (Maltodextrin, starch, whey, lactose…)	9	3	0	2	1						1	
		Total		33	7	0	6	1	3					1	
9	Cocoa and Chocolate products NFDM (375 g)	a	Cocoa Powder	14	0	0	0			0	/	0	/	0	
		b	Chocolates	15	0	0	0			0		0		0	
		c	Raw Ingredients	14	1	0	0			1		1		1	
		Total		43	1	0	0			1	3	1	6	1	
	Cocoa and Chocolate products BPW (375 g)	a	Cocoa Powder	17	2	0	3	-1						-1	
		b	Chocolates	16	2	0	1	1	/					1	
		c	Raw Ingredients	17	2	0	3	-1						-1	
		Total		50	6	0	7	-1	3					-1	
10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	16	2	1	5	-2	/					-2	
		b	Wet Pet Food	15	3	0	4	-1						-1	

Category		Type		N+	ND	PPND	PD	Unpaired (ND+PPND)-PD	Unpaired AL	Paired (ND+PPND)-PD	Paired AL	Paired ND+PPND+PD	Paired AL	Combined (ND+PPND)-PD	Combined AL
		Total		31	5	1	9	-3	3					-3	
11	Meat Products (375 g) (8Hour)	a	Raw	13	3	0	2	1	/					1	
		b	Seasoned & Marinated	12	2	0	4	-2						-2	
		c	Frozen	11	3	0	5	-2						-2	
		Total		36	8	0	11	-3	3					-3	
	Meat Products (375 g) (24Hour)	a	Raw	13	2	0	2	0	/					0	
		b	Seasoned & Marinated	13	2	0	5	-3						-3	
		c	Frozen	11	3	0	5	-2						-2	
		Total		37	7	0	12	-5	3					-5	
12	Meat Products (25 g) (8Hour)	a	Raw	10	4	0	3	1	/					1	
		b	Seasoned & Marinated	10	4	0	4	0						0	
		c	Frozen	10	3	0	3	0						0	
		Total		30	11	0	10	1	3					1	
	Meat Products (25 g) (24Hour)	a	Raw	10	4	0	3	1	/					1	
		b	Seasoned & Marinated	10	4	0	4	0						0	
		c	Frozen	10	3	0	3	0						0	
		Total		30	11	0	10	1	3					1	
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3	/					-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3						-3	
		c	Related Products/Ingredients	11	1	0	1	0						0	
		Total		35	1	0	7	-6	3					-6	
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3	/					-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3						-3	
		c	Related Products/Ingredients	11	1	0	1	0						0	
		Total		35	1	0	7	-6	3					-6	
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1	/					-1	
		b	Vegetables	12	4	0	4	0						0	
		c	Fruit & Juices	11	4	0	2	2						2	
		Total		37	10	0	9	1	3					1	
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1	/					-1	
		b	Vegetables	12	4	0	4	0						0	
		c	Fruit & Juices	11	4	0	2	2						2	
		Total		37	10	0	9	1	3					1	
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed	14	2	0	0	2	/					2	
		b	Livestock Feed	10	4	0	3	1						1	
		c	Ingredients of Feed Products	10	0	0	0	0						0	
		Total		34	6	0	3	3	3					3	
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	0	0	0			0		0		0	
		b	Wet Pet Food	15	0	0	1			-1		1		-1	
		Total		30	0	0	1			-1	3	1	6	-1	
A: All products + All Extension Categories Minimum Enrichment Time				**503**	**71**	**5**	**80**							**-4**	
B: All products + All Extension Categories Minimum Enrichment Time				**505**	**70**	**6**	**82**							**-6**	
C: All products + All Extension Categories Maximum Enrichment Time				**505**	**71**	**4**	**82**							**-7**	
D: All products + All Extension Categories Maximum Enrichment Time				**507**	**70**	**5**	**84**							**-9**	7
E: All products + All Extension Categories Minimum Enrichment Time				**510**	**76**	**5**	**87**							**-6**	
F: All products + All Extension Categories Minimum Enrichment Time				**512**	**75**	**6**	**89**							**-8**	
G: All products + All Extension Categories Maximum Enrichment Time				**512**	**76**	**4**	**89**							**-9**	

Mainly unpaired data studies have been run. The ISO 16140-2:2016 provides the Acceptability Limits for a total of up to 8 categories, while 16 categories are possibly combined for the alternative method. However, all categories analyzed (ND+PPND) - ND and (ND+PPND) + PD are lower than the acceptability limit (AL) set up for 8 categories for unpaired data study.

The observed values for ((ND + PPND) - PD) and for ND + PPND + PD meet the Acceptability Limit (observed values ≤ AL) for the individual categories for the three PCR instruments (PikoReal, 7500 Fast, QS5).

The observed values for ((ND + PPND) - PD) meet the Acceptability Limit (observed values ≤ AL) for all the combined categories for the three PCR instruments (PikoReal, 7500 Fast, and QS5).

3.1.6 Confirmation protocols

Two confirmation protocols were tested during the study: direct streaking onto *Brilliance*™ Salmonella Agar and subculture in RVS broth for 24 h±3 h at 41.5±°C prior streaking onto *Brilliance* Salmonella Agar. The expected confirmation pathway according to the ISO 16140-2-2016 standard (clause 5.1.3.3), is covered with the sub-culture in RVS: this additional pathway diverts a portion of the alternative method's incubated enrichment to that of the reference method. Again, the two confirmation tests, i.e. latex agglutination and biochemical galleries, were run on the observed characteristic colonies. While this pathway is systematically available in a paired study, this additional confirmation flow was conducted specifically for the unpaired data studies. The differences observed for all previously validated products are given in **Table 27** for the PikoReal PCR Instrument and in **Table 28** for the 7500 Fast PCR instrument.

Table 27 - Difference observed for PikoReal PCR Instrument

Category		Confirmation protocol	PikoReal PCR instrument							
			PA	NA	PD	ND	PPND	PPNA	Total	PA+PD
1	Meat	Brilliance	31	37	0	2	1	1	72	31
		RVS/Brilliance	32	37	0	2	0	1	72	32
2	Milk and dairy products-BPW	Brilliance	21	32	4	4	1	0	62	25
		RVS/Brilliance	21	32	4	4	1	0	62	25
	Milk and dairy products-OBS	Brilliance	21	30	6	5	0	0	62	27
		RVS/Brilliance	21	30	6	5	0	0	62	27
3	Seafood and vegetables	Brilliance	30	45	0	0	2	0	77	30
		RVS/Brilliance	32	45	0	0	0	0	77	32
4	Egg products	Brilliance	31	33	0	0	0	2	66	31
		RVS/Brilliance	31	33	0	0	0	2	66	31
5	Raw beef 8h	Brilliance	34	47	2	3	4	1	91	36
		RVS/Brilliance	38	47	3	3	0	0	91	41
	Raw beef 24h	Brilliance	38	46	3	2	1	1	91	41
		RVS/Brilliance	40	46	4	1	0	0	91	44
6	Infant formula	Brilliance	32	30	0	2	0	0	64	32
		RVS/Brilliance	32	30	0	2	0	0	64	32
7	Pet food	Brilliance	27	36	2	1	1	2	69	29
		RVS/Brilliance	28	36	2	1	0	2	69	30
A		**Brilliance**	**206**	**260**	**8**	**12**	**9**	**6**	**501**	**214**
		RVS/Brilliance	**214**	**260**	**9**	**12**	**1**	**5**	**501**	**223**
B		**Brilliance**	**206**	**258**	**10**	**13**	**8**	**6**	**501**	**216**
		RVS/Brilliance	**214**	**258**	**11**	**13**	**0**	**5**	**501**	**225**
C		**Brilliance**	**210**	**255**	**9**	**11**	**6**	**6**	**501**	**219**
		RVS/Brilliance	**216**	**255**	**10**	**10**	**1**	**5**	**501**	**226**
D		**Brilliance**	**210**	**253**	**11**	**12**	**5**	**6**	**501**	**221**
		RVS/Brilliance	**216**	**253**	**12**	**11**	**0**	**5**	**501**	**228**

Table 28 - Difference observed for 7500 Fast PCR instrument

Category	Confirmation protocol	7500 Fast							
		PA	NA	PD	ND	PPND	PPNA	Total	PA+PD
Meat	Brilliance	23	35	7	4	3	0	72	30
	RVS/Brilliance	26	35	7	4	0	0	72	33
Dairy-BPW	Brilliance	21	28	4	4	1	0	58	25
	RVS/Brilliance	21	28	4	4	1	0	58	25
Dairy OBS	Brilliance	21	27	5	5	0	0	58	26
	RVS/Brilliance	21	27	5	5	0	0	58	26
Powdered infant formula	Brilliance	33	30	0	1	0	0	64	33
	RVS/Brilliance	33	30	0	1	0	0	64	33
Vegetables	Brilliance	19	33	5	4	3	0	64	24
	RVS/Brilliance	22	33	5	3	1	0	64	27
Seafood products	Brilliance	22	33	3	5	1	0	64	25
	RVS/Brilliance	22	33	3	5	1	0	64	25
Raw beef 8h	Brilliance	20	31	5	5	0	0	61	25
	RVS/Brilliance	20	31	5	5	0	0	61	25
Raw beef 24h	Brilliance	21	31	5	4	0	0	61	26
	RVS/Brilliance	21	31	5	4	0	0	61	26
Environmental samples	Brilliance	27	39	0	3	0	0	69	27
	RVS/Brilliance	27	39	0	3	0	0	69	27
Infant formula (300 g)	Brilliance	20	31	6	6	0	0	63	26
	RVS/Brilliance	21	30	6	6	0	0	63	27
A	**Brilliance**	**163**	**227**	**27**	**27**	**7**	**0**	**451**	**190**
	RVS/Brilliance	**170**	**226**	**27**	**26**	**2**	**0**	**451**	**197**
B	**Brilliance**	**163**	**226**	**28**	**28**	**6**	**0**	**451**	**191**
	RVS/Brilliance	**170**	**225**	**28**	**27**	**1**	**0**	**451**	**198**
C	**Brilliance**	**164**	**227**	**27**	**26**	**7**	**0**	**451**	**191**
	RVS/Brilliance	**171**	**226**	**27**	**25**	**2**	**0**	**451**	**198**
D	**Brilliance**	**164**	**226**	**28**	**27**	**6**	**0**	**451**	**192**
	RVS/Brilliance	**171**	**225**	**28**	**26**	**1**	**0**	**451**	**199**

A subculture onto RVS prior to streaking onto *Brilliance* Salmonella Agar allows in most of the cases a better recovery of the *Salmonella* strains. The same data sets were observed for the QS5 PCR Instrument. Note that no difference between direct streaking and a sub-culture in RVS prior to streaking was observed for the cocoa and chocolate products.

3.1.7 PCR inhibition

No inhibition was observed in the extension study (2023) or with the cocoa and chocolate products.

Inhibition was observed in previous extensions. For the PikoReal PCR instrument a total of 12 (1.2%) inhibition results were observed. For the 7500 Fast PCR instrument a total of 8 (0.8%) inhibition results were observed. For the QS5 PCR instrument a total of 2 (0.2%) inhibition results were observed. A total of 10 inhibition results were observed in dairy products, with only 7 different dairy matrixes as some samples were tested with two enrichment protocols. For these samples, a 1/5 or 1/10 dilution was applied.

The inhibition results observed for all previously validated products are listed in **Table 29** for the PikoReal, **Table 30** for the 7500 Fast and **Table 31** for the QS5 PCR instruments.

Table 29 - PCR inhibitions - PikoReal PCR Instrument

Sample	Product	PCR Result
4	Raw poultry meat	i/-
2923	Sandwich (72 h)	i/36.01
492	Raw milk (BPW+Novobiocin)	i/i/- *
492	Raw milk (ONE Broth-Salmonella + Novobiocin)	i/-/-
421	Raw milk (BPW+Novobiocin)	i/i/- *
421	Raw milk (ONE Broth-Salmonella + Novobiocin)	i/i/- *
422	Raw sheep milk (BPW+Novobiocin)	i/-
780	Ice cream (BPW + Novobiocin) (72 h)	i/i/- *
486	Pasteurised milk cheese (72 h)	i/i/-*
489	Pasteurised cream (ONE Broth-Salmonella + Novobiocin 72 h)	i/+ (31.10)
489	Pasteurised cream (BPW + Novobiocin 72 h)	i/-
492	Raw milk (ONE Broth-Salmonella + Novobiocin 72 h)	i/-/-
	Number of inhibitions	**12**
	Total lysates tested	**981**
	Percentage of inhibition	**1.2 %**

With (..): Ct value
*: 1/10 dilution of the enrichment broth
i: PCR inhibition

Table 30 – PCR inhibitions - 7500 Fast PCR instrument

Sample n°	Product	PCR Result
695	Rinsing water (72 h)	i/-
492	Raw milk (BPW+Novobiocin)	i/i/- *
339	Caseinate (BPW+Novobiocin) (72 h)	i/+ (21.20)
2235	Ground beef (9 h)	i/-
444	Frozen Carpaccio (24 h)	i/-
862	Veal meat (24 h)	i/+ (31.29)
6465	Seafood cocktail (BPW+Novobiocin)	i/-*
6481	Salad mixture (BPW+Novobiocin)	i/+(36.15)*
	Number of inhibitions	**8**
	Number of lysates tested	**1005**
	Percentage of inhibition	**0.8%**

Table 31 – PCR inhibitions - QS5 PCR instrument

Sample n°	Product	PCR Result
986	Infant formula with probiotics (25 g)	i/-*
425	Chicken meat (72 h)	i/-*
	Number of inhibitions	**2**
	Number of lysates tested	**1005**
	Percentage of inhibition	**0.2%**

3.1.8 Enrichment broth storage at 5 ± 3 °C for 72 h

For the extension study (2023), enrichment broth (pre-warmed (37±1 or 41.5°C) BPW ISO) was stored at 2-8°C for 72 h, and all confirmed positive and discrepant samples were tested again, with the exception of the pet and livestock feed types of the Animal Feed category.

For all previously validated products a total of 303 and 299 samples were tested again after enrichment broth storage for 72 h at 5°C ± 3°C respectively for the PikoReal, 7500 Fast, and QS5 PCR instruments. The changes observed are presented in **Table 32** through **34**.

Table 32 - Evolution of results - PikoReal PCR Instrument

Category		Protocol	N°sample	Before storage	After storage	Type
1	Meat products	BPW	10	**ND**	PA	c
2	Milk and dairy products	ONE-Broth-Salmonella + 12mg/L Novobiocin	489	**ND**	PA	a
5	Raw beef meat 8 h	BPW 8 h	2667	PA	**ND**	b
			5310	PA	**ND**	b
6	Infant formula	BPW	1104	PA	**PPND**	b

Table 33 - Evolution of results - 7500 Fast PCR Instrument

Category		Protocol	N°sample	Before storage	After storage	Type
2	Milk and dairy products	OBS+12mg/L Novobiocin	492	**NA**	**PD**	a
3	Infant formula	BPW	987	**PA**	**ND**	b
			1104	**PA**	**PPND**	b
4	Vegetables	BPW+Novobiocin	**6689**	**PA**	**ND**	a
			6694	**ND**	**PA**	a
8	Milk powder, infant formula with and without probiotics (375 g)	BPW+Vancomycin	**342**	**ND**	**PA**	a
			958	**PA**	**ND**	a
10	Pet Food	1:6 with pre-warmed (37±1°C) BPW ISO	**109**	**PPND**	**ND**	a
			247	**PA**	**ND**	b
15	Animal Feed	1:10 ratio of BPW ISO with novobiocin at 12 mg/L	**2319045**	**PA**	**ND**	c

Table 34 - Evolution of results – QS5 PCR Instrument

Category		Protocol	N°sample	Before storage	After storage	Type
2	Milk and dairy products	CBS+12mg/L Novobiocin	489	PPND	PA	a
3	Infant formula	BPW	987	PA	ND	b
			1104	PA	PPND	b
4	Vegetables	BPW+Novobiocin	6693	PD	NA	a
			6696	ND	PA	b
5	Seafood products	BPW+Novobiocin	6467	PD	NA	a
6	Raw beef meat	BPW (9 h)	566	PA	ND	a
			440	NA	PD	b
7	Environmental samples	BPW	7728	PA	ND	c
8	Milk powder, infant formula with and without probiotics (375 g)	BPW+Vancomycin	342	ND	PA	a
			958	ND	PA	a
10	Pet Food	1:6 with pre-warmed (37±1°C) BPW ISO	109	PPND	ND	a
			247	PA	ND	b
15	Animal Feed	1:10 ratio of BPW ISO with novobiocin at 12 mg/L	2319045	PA	ND	c

The analysis of discordant results after enrichment broth storage is presented in **Tables 35 to 37**.

Table 35 - Analysis of discordant results after 72 h storage of the enrichment broth - PikoReal PCR Instrument

Category		Type		N+	ND	PPND	PD	Unpaired (ND+PPND)-PD	Unpaired AL	Paired (ND+PPND)-PD	Paired AL	Paired ND+PPND+PD	Paired AL	Combined (ND+PPND)-PD	Combined AL
1	Meat products BPW	a	Unprocessed	9	0	0	0			0	/	0	/	0	
		b	Processed	12	1	0	0			1		1		1	
		c	RTE and RTRH products	13	0	0	0			0		0		0	
		Total		34	1	0	0			1	3	1	6	1	
2	Milks and dairy products BPW Novo	a	Pasteurized products	11	2	0	3	-1	/					-1	
		b	Raw products	8	2	0	0	2						2	
		c	Ingredients and low moisture products	11	1	0	1	0						0	
		Total		30	5	0	4	1	3					1	
	Milks and dairy products OBS Novo	a	Pasteurized products	11	0	0	3	-3	/					-3	
		b	Raw products	9	2	0	1	1						1	
		c	Ingredients and low moisture products	11	2	0	1	1						1	
		Total		31	4	0	5	-1	3					-1	
3	Seafood and vegetables BPW	a	Fresh, raw, frozen products	12	0	0	0			0	/	0	/	0	
		b	Heat treated products	11	0	0	0			0		0		0	
		c	Composite foods	9	0	0	0			0		0		0	
		Total		32	0	0	0			0	3	0	6	0	
4	Egg products BPW	a	Egg powders	11	0	0	0			0	/	0	/	0	
		b	Liquid egg products	12	0	0	0			0		0		0	
		c	Egg based products	7	0	0	0			0		0		0	
		Total		30	0	0	0			0	3	0	6	0	
5	Raw beef meats BPW 8h	a	Fresh meats	26	3	0	2	1	/					1	
		b	Frozen meats	7	1	0	1	0						0	
		c	Seasoned meats	11	1	0	0	1						1	
		Total		44	5	0	3	2	3					2	
	Raw beef meats BPW 24h	a	Fresh meats	26	1	0	2	-1	/					-1	
		b	Frozen meats	8	0	0	2	-2						-2	
		c	Seasoned meats	11	1	0	0	1						1	
		Total		45	2	0	4	-2	3					-2	
6	Infant formula	a	Infant formula without probiotics	16	0	0	0			0	/	0	/	0	
		b	Infant formula with probiotics	18	1	2	0			3		3		3	
		Total		34	1	2	0			3	3	3	6	3	
7	Pet food OBS + suppl	a	Raw materials	10	1	0	1	0	/					0	
		b	Low moisture products	10	0	0	1	-1						-1	
		c	High moisture products	11	0	0	0	0						0	
		Total		31	1	0	2	-1	3					-1	
A: All products - Dairy BPW Novo -Raw beef 8h				**235**	**13**	**2**	**9**							**5**	**7**
B: All products - Dairy OBS Novo -Raw beef 8h				**236**	**12**	**2**	**10**							**4**	
C: All products - Dairy BPW Novo -Raw beef 24h				**236**	**10**	**2**	**10**							**2**	
D: All products - Dairy OBS Novo -Raw beef 24h				**237**	**9**	**2**	**11**							**0**	

Table 36 - Analysis of discordant results after storage for 72 h at 5°C ± 3°C – 7500 Fast PCR instrument

Category		Type		N+	ND	PPND	PD	Unpaired		Paired				Combined	
								(ND+PPND)-PD	AL	(ND+PPND)-PD	AL	ND+PPND+PD	AL	(ND+PPND)-PD	AL
1	Meat products	a	Raw meat products (frozen or fresh)	14	2	0	4	-2						-2	
		b	Raw poultry (fresh or frozen)	13	3	0	3	0	/					0	
		c	Raw delicatessen	11	0	0	0	0						0	
		Total		38	5	0	7	-2	3					-2	
2	Milks and dairy products BPW novo	a	Pasteurized products	11	2	0	3	-1						-1	
		b	Raw products	8	1	1	0	2	/					2	
		c	Ingredients and low moisture products	11	1	0	1	0						0	
		Total		30	4	1	4	1	3					1	
	Milks and dairy products OBS novo	a	Pasteurized products	12	0	0	4	-4						-4	
		b	Raw products	9	2	0	1	1	/					1	
		c	Ingredients and low moisture products	11	2	0	1	1						1	
		Total		32	4	0	6	-2	3					-2	
3	Infant formula	a	Infant formula without probiotics	16	0	0	0			0	/	0	/	0	
		b	Infant formula with probiotics	18	1	2	0			3		3		3	
		Total		34	1	2	0			3	3	3	6	3	
4	Vegetables	a	Raw vegetables (fresh, frozen)	10	2	0	0	2						2	
		b	Processed. under atmosphere	8	0	0	2	-2	/					-2	
		c	RTE	13	1	1	3	-1						-1	
		Total		31	3	1	5	-1	3					-1	
5	Seafood products	a	Raw fishery products	9	1	0	0	1						1	
		b	RTRH	10	0	0	0	0	/					0	
		c	RTE	11	4	0	3	1						1	
		Total		30	5	0	3	2	3					2	
6	Raw beef meats 9 h	a	Fresh meats	10	1	0	1	0						0	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		30	5	0	5	0	3					0	
	Raw beef meats 24 h	a	Fresh meats	10	0	0	1	-1						-1	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		30	4	0	5	-1	3					-1	
7	Environmental samples	a	Dusts and Residues	9	2	0	0			2	/	2	/	2	
		b	Cleaning and Process Waters	11	0	0	0			0		0		0	
		c	Surface samples	10	1	0	0			1		1		1	
		Total		30	3	0	0			3	3	3	6	3	
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	a	Milk powder, Infant formula and infant cereals without probiotics	11	1	0	1	0						0	
		b	Infant formula and infant cereals with probiotics	13	2	0	3	-1	/					-1	
		c	Ingredients (Maltodextrin, starch, whey, lactose...)	9	3	0	2	1						1	
		Total		33	6	0	6	0	3					0	
9	Cocoa and chocolate products NFDM (375g)	a	Cocoa Powder	14	0	0	0			0	/	0	/	0	
		b	Chocolates	15	0	0	0			0		0		0	
		c	Raw Ingredients	14	0	0	0			0		0		0	
		Total		43	0	0	0			0	3	0	6	0	
	Cocoa and chocolate products BPW (375g)	a	Cocoa Powder	17	3	0	3	0						0	
		b	Chocolates	17	2	0	2	0	/					0	
		c	Raw Ingredients	17	2	0	3	-1						-1	
		Total		51	7	0	8	-1	3					-1	

10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	16	3	0	5	-2	/					-2	
		b	Wet Pet Food	15	4	0	4	0						0	
		Total		31	7	0	9	-2	3					-2	
11	Meat Products (375 g) (8Hour)	a	Raw	13	3	0	2	1	/					1	
		b	Seasoned & Marinated	12	2	0	4	-2						-2	
		c	Frozen	11	3	0	5	-2						-2	
		Total		36	8	0	11	-3	3					-3	
	Meat Products (375 g) (24Hour)	a	Raw	13	2	0	2	0	/					0	
		b	Seasoned & Marinated	13	2	0	5	-3						-3	
		c	Frozen	11	3	0	5	-2						-1	
		Total		37	7	0	12	-5	3					-5	
12	Meat Products (25 g) (8Hour)	a	Raw	10	4	0	3	1	/					1	
		b	Seasoned & Marinated	10	4	0	4	0						0	
		c	Frozen	10	3	0	3	0						0	
		Total		30	11	0	10	1	3					1	
	Meat Products (25 g) (24Hour)	a	Raw	10	4	0	3	1	/					1	
		b	Seasoned & Marinated	10	4	0	4	0						0	
		c	Frozen	10	3	0	3	0						0	
		Total		30	11	0	10	1	3					1	
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3	/					-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3						-3	
		c	Related Products/Ingredients	11	1	0	1	0						0	
		Total		35	1	0	7	-6	3					-6	
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3	/					-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3						-3	
		c	Related Products/Ingredients	11	1	0	1	0						0	
		Total		35	1	0	7	-6	3					-6	
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1	/					-1	
		b	Vegetables	12	4	0	4	0						0	
		c	Fruit & Juices	11	4	0	2	2						2	
		Total		37	10	0	9	1	3					1	
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1	/					-1	
		b	Vegetables	12	4	0	4	0						0	
		c	Fruit & Juices	11	4	0	2	2						2	
		Total		37	10	0	9	1	3					1	
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed						/						
		b	Livestock Feed												
		c	Fruit and Juices	9	0	0	0	0						0	
		Total		9	0	0	0	0	3					0	
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	0	0	0			0	/	0	/	0	
		b	Wet Pet Food	15	0	0	1			-1		1		-1	
		Total		30	0	0	1			-1	3	1	6	-1	
A: All products + All Extension Categories Minimum Enrichment Time				**573**	**66**	**4**	**69**							-4	7
B: All products + All Extension Categories Minimum Enrichment Time				**576**	**66**	**3**	**69**							-7	
C: All products + All Extension Categories Maximum Enrichment Time				**573**	**64**	**4**	**67**							-7	
D: All products + All Extension Categories Maximum Enrichment Time				**576**	**64**	**3**	**67**							-10	
E: All products + All Extension Categories Minimum Enrichment Time				**596**	**73**	**4**	**76**							-5	
F: All products + All Extension Categories Minimum Enrichment Time				**599**	**73**	**3**	**76**							-8	
G: All products + All Extension Categories Maximum Enrichment Time				**596**	**71**	**4**	**74**							-8	
H: All products + All Extension Categories Maximum Enrichment Time				**599**	**71**	**3**	**74**							-11	

Table 37 - Analysis of discordant results after storage for 72 h at 5°C ± 3°C – QS5 PCR instrument

Category		Type		N+	ND	PPND	PD	Unpaired (ND+PPND)-PD	AL	Paired (ND+PPND)-PD	AL	Paired ND+PPND+PD	AL	Combined (ND+PPND)-PD	AL
1	Meat products	a	Raw meat products (frozen or fresh)	14	2	0	4	-2						-2	
		b	Raw poultry (fresh or frozen)	13	3	0	3	0	/					0	
		c	Raw delicatessen	11	0	0	0	0						0	
		Total		38	5	0	7	-2	3					-2	
2	Milks and dairy products BPW novo	a	Pasteurized products	11	2	0	3	-1						-1	
		b	Raw products	8	1	1	0	2	/					2	
		c	Ingredients and low moisture products	11	1	0	1	0						0	
		Total		30	4	1	4	1	3					1	
	Milks and dairy products OBS novo	a	Pasteurized products	12	0	0	4	-4						-4	
		b	Raw products	9	2	0	1	1	/					1	
		c	Ingredients and low moisture products	11	2	0	1	1						1	
		Total		32	4	0	6	-2	3					-2	
3	Infant formula	a	Infant formula without probiotics	16	0	0	0			0	/	0	/	0	
		b	Infant formula with probiotics	18	2	1	0			3		3		3	
		Total		34	2	1	0			3	3	3	6	3	
4	Vegetables	a	Raw vegetables (fresh, frozen)	10	1	0	0	1						1	
		b	Processed, under atmosphere	8	0	0	2	-2	/					-2	
		c	RTE	13	2	0	3	-1						-1	
		Total		31	3	0	5	-2	3					-2	
5	Seafood products	a	Raw fishery products	9	1	0	0	1						1	
		b	RTRH	10	0	0	0	0	/					0	
		c	RTE	11	3	1	3	1						1	
		Total		30	4	1	3	2	3					2	
6	Raw beef meats 9 h	a	Fresh meats	10	1	0	1	0						0	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	2	0	1	1						1	
		Total		30	5	0	5	0	3					0	
	Raw beef meats 24 h	a	Fresh meats	10	0	0	1	-1						-1	
		b	Frozen meats	11	2	0	3	-1	/					-1	
		c	Seasoned meats	9	1	1	1	1						1	
		Total		30	3	1	5	-1	3					-1	
7	Environmental samples	a	Dusts and Residues	9	2	0	0			2	/	2	/	2	
		b	Cleaning and Process Waters	11	0	0	0			0		0		0	
		c	Surface samples	10	1	0	0			1		1		1	
		Total		30	3	0	0			3	3	3	6	3	
8	Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)	a	Milk powder, Infant formula and infant cereals without probiotics	11	0	0	1	-1						-1	
		b	Infant formula and infant cereals with probiotics	13	2	0	3	-1	/					-1	
		c	Ingredients (Maltodextrin, starch, whey, lactose...)	9	3	0	2	1						1	
		Total		33	5	0	6	-1	3					-1	
9	Cocoa and chocolate products NFDM (375g)	a	Cocoa Powder	14	0	0	0			0	/	0	/	0	
		b	Chocolates	15	0	0	0			0		0		0	
		c	Raw Ingredients	14	1	0	0			1		1		1	
		Total		43	1	0	0			1	3	1	6	1	
	Cocoa and chocolate products BPW (375g)	a	Cocoa Powder	17	2	0	3	-1						-1	
		b	Chocolates	16	2	0	1	1	/					1	
		c	Raw Ingredients	17	2	0	3	-1						-1	
		Total		51	6	0	7	-1	3					-1	

10	Pet Food (375 g) (1:6 ratio)	a	Dry Pet Food	16	3	0	5	-2	/			-2	
		b	Wet Pet Food	15	4	0	4	0				0	
		Total		31	7	0	9	-2	3			-2	
11	Meat Products (375 g) (8Hour)	a	Raw	13	3	0	2	1	/			1	
		b	Seasoned & Marinated	12	2	0	4	-2				-2	
		c	Frozen	11	3	0	5	-2				-2	
		Total		36	8	0	11	-3	3			-3	
	Meat Products (375 g) (24Hour)	a	Raw	13	2	0	2	0	/			0	
		b	Seasoned & Marinated	13	2	0	5	-3				-3	
		c	Frozen	11	3	0	5	-2				-2	
		Total		37	7	0	12	-5	3			-5	
12	Meat Products (25 g) (8Hour)	a	Raw	10	4	0	3	1	/			1	
		b	Seasoned & Marinated	10	4	0	4	0				0	
		c	Frozen	10	3	0	3	0				0	
		Total		30	11	0	10	1	3			1	
	Meat Products (25 g) (24Hour)	a	Raw	10	4	0	3	1	/			1	
		b	Seasoned & Marinated	10	4	0	4	0				0	
		c	Frozen	10	3	0	3	0				0	
		Total		30	11	0	10	1	3			1	
13	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (18 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3	/			-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3				-3	
		c	Related Products/Ingredients	11	1	0	1	0				0	
		Total		35	1	0	7	-6	3			-6	
	Powdered Infant Formula and Cereals with and without probiotics including ingredients (e.g. caseinates) (375 g) (26 Hour)	a	Powdered Infant Formula (no Probiotics)	12	0	0	3	-3	/			-3	
		b	Powdered Infant Formula (with Probiotics)	12	0	0	3	-3				-3	
		c	Related Products/Ingredients	11	1	0	1	0				0	
		Total		35	1	0	7	-6	3			-6	
14	Fresh and Processed Vegetables and Fruit (375 g) (10 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1	/			-1	
		b	Vegetables	12	4	0	4	0				0	
		c	Fruits and Juices	11	4	0	2	2				2	
		Total		37	10	0	9	1	3			1	
	Fresh and Processed Vegetables and Fruit (375 g) (24 Hour)	a	Sprouted Seeds & Leafy Greens	14	2	0	3	-1	/			-1	
		b	Vegetables	12	4	0	4	0				0	
		c	Fruits and Juices	11	4	0	2	2				2	
		Total		37	10	0	9	1	3			1	
15	Animal Feed (150 g) (20 Hour)	a	Pet Feed						/				
		b	Livestock Feed										
		c	Ingredients of Feed Products	9	0	0	0	0				0	
		Total		9	0	0	0	0	3			0	
16	Pet Food (375 g) (1:10 ratio)	a	Dry Pet Food	15	0	0	0			0	/	0	/	0	
		b	Wet Pet Food	15	0	0	1			-1		1		-1	
		Total		30	0	0	1			-1	3	1	6	-1	
A: All products + All Extension Categories Minimum Enrichment Time				542	68	4	76					-4	
B: All products + All Extension Categories Minimum Enrichment Time				545	68	3	78					-7	
C: All products + All Extension Categories Maximum Enrichment Time				542	65	5	77					-7	
D: All products + All Extension Categories Maximum Enrichment Time				545	65	4	79					-10	7
E: All products + All Extension Categories Minimum Enrichment Time				561	73	4	83					-6	
F: All products + All Extension Categories Minimum Enrichment Time				564	73	3	85					-9	
G: All products + All Extension Categories Maximum Enrichment Time				561	70	5	84					-9	

The observed values for ((ND + PPND) - PD) and for ND + PPND + PD meet the Acceptability Limit (observed values ≤ AL) for the individual categories for the three PCR instruments (PikoReal, 7500 Fast, QS5).

The observed values for ((ND + PPND) - PD) meet the Acceptability Limit (observed values ≤ AL) for all the combined categories for the three PCR instruments (PikoReal, 7500 Fast, QS5).

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.
The RLOD is defined as the ratio of the alternative and reference methods:

$$RLOD = \frac{LOD_{alt}}{LOD_{ref}}$$

3.2.1 Experimental design

One sample type and one relevant target micro-organism for this sample type was chosen in RLOD study, as shown in **Tables 38 and 39.**

A total of 30 test portions were analyzed per RLOD. All test portions will be compared to the ISO 6579-1:2017 reference standard and carried out according to EN ISO 16140-2:2016. A total plate count was performed on the day of analysis to determine the total microbial load.

Table 38 - Tested (matrix/strain) pairs for the RLOD determination - PikoReal PCR Instrument

Categories		Matrices	Strains	Protocol
1	**Meat products including poultry meats**	Poultry meat	*Salmonella* Braenderup Ad915	BPW for 20 - 24 h at 34-38°C
2	**Dairy products**	Raw milk	*Salmonella* Montevideo 606	BPW + 12mg/l Novobiocin for 20-24 h at 37°C ±1°C and ONE Broth *Salmonella* + 12 mg/l Novobiocin for 20 - 24 h at 34-38°C
3	**Seafood and vegetables**	Spinach	*Salmonella* Virchow Ad1721	BPW for 20 - 24 h at 34-38°C
4	**Egg products**	Liquid egg	*Salmonella* Enteritidis 10	BPW for 20 - 24 h at 34-38°C
5	**Raw beef meats with and without aromatics**	Ground beef	*Salmonella* Typhimurium A00C060	Pre-warmed BPW for 8 - 24 h at 41.5°C
6	**Infant formula**	Infant formula with probiotics	*Salmonella* Anatum Ad298	BPW for 16 - 20 h at 34-38°C
7	**Pet food**	Balls for dogs	*Salmonella* Derby 630	ONE Broth *Salmonella* + ONE Broth *Salmonella* supplement for 20 - 24 h at 34-38°C

Table 39 - Tested (matrix/strain) pairs for the RLOD determination - 7500 Fast and QS5 PCR instruments

	Categories	Matrices	Strains	Storage conditions prior to analysis	Protocol
1	**Meat products**	Raw chicken meat	*Salmonella* Bredeney 975	48 h at 3°C ± 2°C	BPW + 12 mg/l Novobiocin for 20 - 24 h at 34-38°C
2	**Milk and dairy products**	Raw milk	*Salmonella* Ohio Ad1482	48 h at 3°C ± 2°C	BPW + 12 mg/l Novobiocin for 20 - 24 h at 34-38°C ONE Broth-*Salmonella* + 12 mg Novobiocin for 20 - 24 h at 34-38°C
3	**Infant formula**	Infant formula with probiotics	*Salmonella* Anatum Ad298	1 - 2 weeks at room temperature	BPW for 16 - 20 h at 34-38°C
4	**Vegetables**	Frozen spinach	*Salmonella* Virchow Ad1721	1 week at -20°C	BPW + 12 mg/l Novobiocin for 20 - 24 h at 34-38°C
5	**Seafood**	Fish terrine	*Salmonella* Derby Ad1093	48 h at 3°C ± 2°C	BPW + 12 mg/l Novobiocin for 20 - 24 h at 34-38°C
6	**Raw beef meats**	Ground beef	*Salmonella* Typhimurium A00C060	48 h at 3°C ± 2°C	Pre-warmed BPW for 9 h at 34-38°C Pre-warmed BPW for 24 h at 34-38°C
7	**Production environmental samples**	Process water	*Salmonella* Livingstone A00L058	48 h at 3°C ± 2°C	BPW for 20 - 24 h at 34-38°C
8	**Milk powder. Infant formula and infant cereals with and without probiotics including ingredients (375 g)**	Infant formula with probiotics	*S.* Mbandaka Ad1810	Lyophilized strain 2 weeks at ambiant temperature	BPW + vancomycin 18 – 22 h at 34-38°C
9	**Cocoa and chocolate products (375g)**	Cocoa Powder	*Salmonella* Typhimurium ATCC 13311	Lyophilized strain 2 weeks at ambiant temperature	NNFDM for 20-28h at 34-38°C Pre-Warmed BPW for 22-30h at 34-38°C
10	**Pet Food (375 g) (1:6 ratio)**	Pâté	*Salmonella* Typhimurium QL 11007- 2	Heat stressed at 50°C for 10 minutes; Held at 2 -8°C for 48-72 h	1:6 with pre-warmed (37±1°C) BPW ISO for 20-28h at 34-38°C
11	**Meat Products (375 g)**	Ground beef	*Salmonella* Dublin UPENN STS27	Overnight culture; Held at 2 -8°C for 48-72 h	1:5 with pre-warmed (41.5±1°C) BPW ISO for 8-24h at 41.5±1°C
12	**Meat Products (25 g)**	Ground beef	*Salmonella* Enteritidis Cornell S5-415	Overnight culture; Held at 2 -8°C for 48-72 h	1:10 with pre-warmed (41.5±1°C) BPW ISO for 8-24h at 41.5±1°C
13	**Powdered Infant Formula (with and without Probiotics) (375 g)**	Infant formula with probiotics	*Salmonella* Tennessee QL 024.6	Lyophilized culture; Held at 20 – 25 °C for 2 weeks	1:6 with pre-warmed (37±1oC) BPW ISO for 18-26h at 34-38°C
		Infant formula without probiotics	*Salmonella* Tennessee QL 024.6	Lyophilized culture; Held at 20 – 25 °C for 2 weeks	1:6 with pre-warmed (37±1oC) BPW ISO (supplemented with novobiocin (6 mg/L)) for 18-26h at 34-38°C
14	**Fresh and Processed Vegetables and Fruit (375 g)**	Baby spinach	*Salmonella* Newport ATCC 6962	Overnight culture; Held at 2 -8°C for 48-72 h	1:10 with pre-warmed (41.5±1°C) BPW ISO for 10-24h at 41.5±1°C

15	**Animal Feed (150 g)**	Cat Kibble	*Salmonella* Infantis EFG554	Heat stressed at 50°C for 10 minutes; Held at 2 -8°C for 48-72 h	1:10 with BPW ISO supplemented with novobiocin at 12 mg/L for 20-28h at 34-38°C
16	**Pet Food (375 g) (1:10 ratio)**	Pâté	*Salmonella* Schwarzengrund QL 10021.1	Heat stressed at 50°C for 10 minutes; Held at 2 -8°C for 48-72 h	1:10 with pre-warmed (37±1°C) BPW ISO for 20-28h at 34-38°C

3.2.2 Test sample preparations

As outlined previously, three levels of artificial contamination were prepared for each type:

- Negative control level: One uninoculated in order to get 5 test portions.
- Low level: One inoculated usually below 2.0 CFU/sample in order to get 20 test portions providing fractional recovery (5-15 positive results out of 20)
- High level: One inoculated usually between 2.0 and 5.0 CFU/sample in order to get 5 test portions contaminated at a higher level.

A bulk lot of the matrix was inoculated at each level, homogenized and stored as described in Table 13.

3.2.3 Calculation and interpretation of the RLOD

The RLOD calculations were performed using the Excel spread sheet https://standards.iso.org/iso/16140/ (version 06-07-2015) of the international standard as described in EN ISO 16140-2: 2016.

The tabulated raw data for the RLOD study is given in **Appendix 5**.

The data for all possible combinations of categories, sample sizes and protocols with the 7500 Fast and QuantStudio 5 instruments are presented **Tables 40 to 43** for the RLOD calculation according to the ISO 16140-2:2016 standard, and **Tables 44 to 47** for the Wilrich & Wilrich POD-LOG calculation (version 09. 2017-09-23).

Table 40: RLOD on 25 g sample size with the PikoReal PCR instrument

Name	RLOD	RLODL	RLODU	B=ln (RLOD)	Sd(b)	z-Test statistic	p-value	AL
Poultry meat (*Salmonella* Braenderup Ad915)	1.263	0.403	3.955	0.234	0.571	0.409	0.682	1.5
Raw Milk -BPW (*Salmonella* Montevideo 606)	0.871	0.369	2.061	-0.138	0.430	0.320	1.251	2.5
Raw Milk – ONE Broth (*Salmonella* Montevideo 606)	1.336	0.534	3.342	0.289	0.459	0.631	0.528	2.5
Frozen Spinach (*Salmonella* Virchow Ad1721)	1.000	0.528	1.895	0.000	0.320	0.000	1.000	1.5
Liquid egg (*Salmonella* Enteritidis 10)	1.000	0.522	1.917	0.000	0.326	0.000	1.000	1.5
Ground Beef – 9 hours (*Salmonella* Typhirurium A00C060)	0.527	0.223	1.244	-0.640	0.429	1.491	1.864	2.5
Ground Beef – 24 hours (*Salmonella* Typhirurium A00C060)	0.527	0.223	1.244	-0.640	0.429	1.491	1.864	2.5
Powdered Infant Formula (*Salmonella* Anatum Ad298)	1.000	0.473	2.113	0.000	0.374	0.000	1.000	1.5
Balls for dogs (*Salmonella* Derby 630)	2.076	0.982	4.391	0.731	0.375	1.951	0.051	2.5
Combined	**1.000**	**0.776**	**1.289**	**0.000**	**0.127**	**0.000**	**1.000**	/

Table 41: RLOD on 25 g sample size with the 7500 Fast instrument

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Chicken meat	1.629	0.696	3.814	0.488	0.425	1.147	0.251
Raw Milk-BPW	1.000	0.447	2.240	0.000	0.403	0.000	1.000
Raw Milk-OBS	1.336	0.534	3.342	0.289	0.459	0.631	0.528
Powdered Infant Formula	1.000	0.473	2.113	0.000	0.374	0.000	1.000
Frozen Spinach	1.000	0.385	2.599	0.000	0.478	0.000	1.000
Fish Terrine	1.149	0.490	2.695	0.139	0.426	0.326	0.744
Ground Beef (9 hours)	0.554	0.239	1.285	-0.591	0.421	1.404	1.840
Ground Beef (24 hours)	0.554	0.239	1.285	-0.591	0.421	1.404	1.840
Process Water	1.170	0.437	3.132	0.157	0.492	0.320	0.749
Ground Beef (*Salmonella* Enteritidis Cornell S5-415)	0.698	0.235	2.074	-0.359	0.544	0.660	1.491
Combined	**0.955**	**0.735**	**1.241**	**-0.046**	**0.131**	**0.350**	**1.274**

Table 42: RLOD on 25 g sample size with the QuantStudio5 Fast instrument

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Chicken meat	1.629	0.696	3.814	0.488	0.425	1.147	0.251
Raw Milk-BPW	0.761	0.326	1.777	-0.274	0.424	0.645	1.481
Raw Milk-OBS	1.336	0.534	3.342	0.289	0.459	0.631	0.528
Powdered Infant Formula	1.000	0.473	2.113	0.000	0.374	0.000	1.000
Frozen Spinach	1.000	0.385	2.599	0.000	0.478	0.000	1.000
Fish Terrine	1.149	0.490	2.695	0.139	0.426	0.326	0.744
Ground Beef (9 hours)	0.554	0.239	1.285	-0.591	0.421	1.404	1.840
Ground Beef (24 hours)	0.554	0.239	1.285	-0.591	0.421	1.404	1.840
Process Water	1.000	0.420	2.383	0.000	0.434	0.000	1.000
Ground Beef (*Salmonella* Enteritidis Cornell S5-415)	0.698	0.235	2.074	-0.359	0.544	0.660	1.491
Combined	**0.923**	**0.712**	**1.198**	**-0.080**	**0.130**	**0.614**	**1.460**

Table 43: RLOD on 375 g sample size with the 7500 Fast and QuantStudio 5 instrument

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value
Powdered Infant Formula w/Probiotics (*Salmonella* Mbandaka AD1810)	0.195	0.084	0.456	-1.633	0.424	3.853	2.000
Cocoa Powdered (Paired)	1.000	0.466	2.145	0.000	0.382	0.000	1.000
Cocoa Powder (Unpaired)	0.757	0.338	1.695	-0.278	0.403	0.690	1.510
Pate (1:6)	0.621	0.229	1.687	-0.476	0.499	0.953	1.659
Ground Beef (*Salmonella* Dublin UPENN STS27)	0.854	0.319	2.286	-0.157	0.492	0.320	1.251
Infant Formula w/Probiotics (*Salmonella* Tennessee QL 024.6)	0.843	0.297	2.397	-0.170	0.522	0.326	1.256
Infant Formula w/o Probiotics (*Salmonella* Tennessee QL 024.6)	1.000	0.420	2.383	0.000	0.434	0.000	1.000
Baby Spinach (*Salmonella* Newport ATCC 6962)	1.186	0.417	3.371	0.170	0.522	0.326	0.744
Cat Kibble (*Salmonella* Infantis EFG554)	1.152	0.471	2.817	0.142	0.447	0.316	0.752
Pate (1:10) (*Salmonella* Schwarzengrund QL 10021.1)	1.000	0.403	2.48	0.000	0.454	0.000	1.000
Combined	**0.753**	**0.576**	**0.985**	**-0.283**	**0.134**	**2.11**	**1.965**

RLOD was the same for 7500 Fast and QS5 instrument and both enrichment protocols.

Table 44: Wilrich & Wilrich POD-LOD on 25 g sample size with the PikoReal PCR instrument

Strain/matrix pair	Level of Detection at 50% (CFU/ sample size) according to Wilrich & Wilrich test.		Level of Detection at 95% (CFU/ sample size) according to Wilrich & Wilrich test.	
	Candidate	**Reference**	**Candidate**	**Reference**
Poultry Meat	0.7 [0.4;1.5]	0.6 [0.3;1.2]	3.2 [1.6;6.5]	2.6 [1.3;5.3]
Raw Milk - BPW	0.7 [0.4;1.0]	0.7 [0.5;1.1]	3.7 [2.0;6.9]	3.7 [2.0;6.9]
Raw Milk – ONE Broth	0.7 [0.4;1.3]	0.7 [0.5;1.1]	3.1 [1.7;5.6]	3.7 [2.0;6.9]
Frozen Spinach	0.4 [0.3;0.7]	0.4 [0.3;0.7]	1.9 [1.2;3.0]	1.9 [1.2;3.0]
Liquid Egg	0.6 [0.4;1.0]	0.6 [0.4;1.0]	2.6 [1.6;4.3]	2.6 [1.6;4.3]
Ground Beef – 8 h	0.2 [0.1;0.5]	0.5 [0.3;0.9]	1.0 [0.5;2.1]	2.3 [1.2;4.1]
Ground Beef – 24 h	0.2 [0.1;0.5]	0.5 [0.3;0.9]	1.0 [0.5;2.1]	2.3 [1.2;4.1]
Powdered Infant Formula with Probiotics	0.6 [0.4;1.1]	0.6 [0.4;1.1]	2.7 [1.6;4.8]	2.7 [1.6;4.8]
Balls for dogs	0.5 [0.3;1.0]	0.5 [0.3;0.9]	2.3 [1.3;4.2]	2.1 [1.2;3.8]

Table 45: Wilrich & Wilrich POD-LOD on 25 g sample size with the 7500 Fast instrument

Strain/matrix pair	Level of Detection at 50% (CFU/ sample size) according to Wilrich & Wilrich test.		Level of Detection at 95% (CFU/ sample size) according to Wilrich & Wilrich test.	
	Candidate	**Reference**	**Candidate**	**Reference**
Raw Chicken Meat	1.5 [0.8;2.9]	1.0 [0.6;1.7]	6.6 [3.6;12.4]	4.2 [2.4;7.3]
Raw Milk - BPW	0.9 [0.5;1.5]	0.9 [0.5;1.5]	3.7 [2.2;6.4]	3.7 [2.2;6.4]
Raw Milk – ONE Broth	1.1 [0.6;1.9]	0.9 [0.5;1.5]	4.7 [2.7;8.2]	3.7 [2.2;6.4]
Powdered Infant Formula with Probiotics	0.6 [0.4;1.1]	0.6 [0.4;1.1]	2.7 [1.6;4.8]	2.7 [1.6;4.8]
Frozen Spinach	0.9 [0.5;1.5]	0.9 [0.5;1.5]	3.7 [2.1;6.7]	3.7 [2.1;6.7]
Fish Terrine	1.0 [0.5;1.8]	0.9 [0.5;1.6]	4.2 [2.3;7.7]	3.8 [2.1;6.8]
Ground Beef – 9 h	0.9 [0.5;1.5]	1.6 [0.8;3.0]	3.8 [2.2;6.6]	6.9 [3.7;12.9]
Ground Beef – 24 h	0.9 [0.5;1.5]	1.6 [0.8;3.0]	3.8 [2.2;6.6]	6.9 [3.7;12.9]
Process Water	0.8 [0.4;1.4]	0.7 [0.4;1.2]	3.4 [1.9;6.0]	3.0 [1.7;5.3]
Ground Beef (*Salmonella* Enteritidis Cornell S5-415)	1.8[1.02;3.16]	2.3[1.2;4.2]	7.8[4.4;13.7]	9.8[5.4;18.0]

Table 46: Wilrich & Wilrich POD-LOG on 25 g sample size with the QuantStudio 5 instrument

Strain/matrix pair	Level of Detection at 50% (CFU/ sample size) according to Wilrich & Wilrich test.		Level of Detection at 95% (CFU/ sample size) according to Wilrich & Wilrich test.	
	Candidate	**Reference**	**Candidate**	**Reference**
Raw Chicken Meat	1.5 [0.8;2.9]	1.0 [0.6;1.7]	6.6 [3.6;12.4]	4.2 [2.4;7.3]
Raw Milk - BPW	0.7 [0.4;1.2]]	0.9 [0.5;1.5]	3.0 [1.8;5.0]	3.7 [2.2;6.4]
Raw Milk – ONE Broth	1.1 [0.6;1.9]	0.9 [0.5;1.5]	4.7 [2.7;8.2]	3.7 [2.2;6.4]
Powdered Infant Formula with Probiotics	0.6 [0.4;1.1]	0.6 [0.4;1.1]	2.7 [1.6;4.8]	2.7 [1.6;4.8]
Frozen Spinach	0.9 [0.5;1.5]	0.9 [0.5;1.5]	3.7 [2.1;6.7]	3.7 [2.1;6.7]
Fish Terrine	1.0 [0.5;1.8]	0.9 [0.5;1.6]	4.2 [2.3;7.7]	3.8 [2.1;6.8]
Ground Beef – 9 h	0.9 [0.5;1.5]	1.6 [0.8;3.0]	3.8 [2.2;6.6]	6.9 [3.7;12.9]
Ground Beef – 24 h	0.9 [0.5;1.5]	1.6 [0.8;3.0]	3.8 [2.2;6.6]	6.9 [3.7;12.9]
Process Water	0.7 [0.4;1.2]	0.7 [0.4;1.2]	3.0 [1.7;5.3]	3.0 [1.7;5.3]
Ground Beef (*Salmonella* Enteritidis Cornell S5-415)	1.8[1.02;3.16]	2.3[1.2;4.2]	7.8[4.4;13.7]	9.8[5.4;18.0]

Table 47: Wilrich & Wilrich POD-LOG on 375 g sample size with the 7500 Fast instrument and QuantStudio 5 instrument

Strain/matrix pair	Level of Detection at 50% (CFU/ sample size) according to Wilrich & Wilrich test.		Level of Detection at 95% (CFU/ sample size) according to Wilrich & Wilrich test.	
	Candidate	**Reference**	**Candidate**	**Reference**
Powdered Infant Formula with Probiotics	0.9 [0.5;1.6]	5.1 [2.6;9.7]	3.6 [2.2;6.8]	21.9 [11.4;41.9]
Cocoa Powder	0.6 [0.3;1.0]	0.7 [0.4;1.3]	2.4 [1.4;4.2]	3.1 [1.8;5.6]
Pate (1:6)	1.3 [0.8;2.2]	1.8 [1.0;3.3]	5.6 [3.3;9.6]	7.8 [4.4;14.1]
Ground Beef (*Salmonella* Dublin UPENN STS27)	0.6 [0.3;1.1]	0.7 [0.4;1.2]	2.6 [1.5;4.7]	3.0 [1.7;5.3]
Infant Formula w/Probiotics (*Salmonella* Tennessee QL 024.6)	1.6 [0.8;2.8]	1.8 [1.0;3.2]	6.8 [3.9;11.9]	7.6 [4.3;13.7]
Infant Formula w/o Probiotics (*Salmonella* Tennessee QL 024.6)	1.4 [0.8;2.4]	1.4 [0.8;2.4]	6.0 [3.5;10.4]	6.0 [3.5;10.4]
Baby Spinach (*Salmonella* Newport ATCC 6962)	1.0 [0.6;1.8]	0.9 [0.5;1.6]	4.3 [2.4;7.8]	3.8 [2.2;6.8]
Cat Kibble (*Salmonella* Infantis EFG554)	0.7 [0.4;1.3]	0.6 [0.4;1.1]	3.1 [1.8;5.5]	2.8 [1.6;4.9]
Pate (1:10) (*Salmonella* Schwarzengrund QL 10021.1)	1.2 [0.7;2.2]	1.2 [0.7;2.2]	5.3 [3.0;9.3]	5.3 [3.0;9.3]

LOD was the same for the 7500 Fast and QS5 instrument and both enrichment protocols.

3.2.4 Conclusion RLOD study

For all tested matrices, sample sizes, protocols and thermocyclers, the RLOD values meet the acceptability limit of 2.5 for unpaired test portions and 1.5 for paired test portions for all types tested.

The LOD50 according to Wilrich & Wilrich varies from 0.3 CFU to 2.9 CFU considering the lowest and highest limits of the confidence intervals.

4 INCLUSIVITY/EXCLUSIVITY (INITIAL VALIDATION. 2013 AND EXTENSION STUDY. 2017)

4.1 Inclusivity / Exclusivity (initial validation. 2013 and extension study. 2017)

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

One hundred target strains and 30 non-target strains were tested during the initial validation (2013) and the renewal study (2017).

For the initial validation study, 55 target strains were tested with the protocol dedicated to raw beef meats (8 h at 41.5°C) as well as 30 non-target strains. The PCR was run using the PikoReal PCR Instrument.

For the extension study carried out in 2017, 45 *Salmonella* strains were tested with the protocol dedicated to raw beef meats (9 h at 41.5°C) using the 7500 Fast Instrument.

4.1.1 Inclusivity testing

Salmonella strains were cultured in BHI medium at 37°C. Dilutions were done in order to inoculate 10 cells/225 ml BPW. The enrichment step was performed in pre-warmed BPW (41.5°C) for 8 or 9 h at 41.5°C. The alternative method protocol was then performed using the PikoReal PCR Instrument for the initial validation study and using the 7500 Fast PCR instrument for the renewal study.

4.1.2 Exclusivity testing

Negative strains were cultured in BHI Broth at 37°C. Dilutions were realised in order to inoculate 105 cells/ml BPW. The BPW was incubated for 24 h at 37°C. The alternative method was then performed using the PikoReal Instrument for the initial validation study and using the 7500 Fast PCR instrument for the extension study.

In agreement with the AFNOR Technical Committee, it was decided to not run again this study using the QS5 PCR Instrument.

4.1.3 Conclusion

The raw data are given in **Appendix 6.**

The method is specific and selective showing satisfying inclusivity and exclusivity data.

5 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.

5.1 Study organization

Samples were sent to 15 laboratories. The study was done with ground beef samples contaminated by *Salmonella* Typhimurium A00C060. Samples were inoculated and sent on Monday 8th July 2013, as described below:

- 24 blind coded samples (25 g) for analysis of *Salmonella* with the Thermo Scientific SureTect Salmonella species method (red label)
- 24 blind coded samples (25 g) for analysis of *Salmonella* by the ISO 6579 (2002) reference method (blue label).
- 1 ground beef sample (labelled “Sample for Total Count enumeration”) for aerobic mesophilic flora enumeration by ISO 4833 method.
- 1 water flask labelled “Temperature Control” with a temperature probe, which must be incubated simultaneously with the samples during analysis (storage and alternative enrichment incubation).

The analyses were started on Tuesday 9th or Wednesday 10th July 2013.The targeted inoculation levels were:

- 0 CFU/25 g.
- 1 – 10 CFU/25 g.
- 5 – 50 CFU/25 g.

Blinded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses.

The samples were shipped in express (24 h maximum), in isotherm packages. The temperature conditions had to stay lower or equal to 8.4°C during transport, and between 0°C – 8.4°C in the labs.
The *Salmonella* detection was performed by the reference and the alternative methods using the Piko-Real PCR instrument. The lower incubation time was tested for the alternative method. i.e. 8 h at 41.5°C.

The collaborative study instructions were sent 26th June 2013.

5.2 Experimental parameters control

5.2.1 Strain stability and background microflora stability

- **Before inoculation**

In order to detect Salmonella species, the EN ISO 6579 method was performed on five ground beef portions (25 g) before the inoculation. All the results were negative.

- **Sample stability**

Sample stability was checked by inoculating the matrix at 100 CFU/g and 5 CFU/g. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level. *Triplicate* samples were analysed. The aerobic mesophilic flora was also enumerated; the results are given in **Table 48.**

Table 48 - Sample stability

Day	Reference method (detection)			CFU/g (XLD)			Aerobic mesophilic flora (CFU/g)
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	
Day 0	+	+	+	200	220	240	800
Day 1	+	+	+	220	210	320	690
Day 2	+	+	+	210	230	280	1600

No evolution was observed during storage at 4°C.

5.2.2 Contamination levels

The contamination levels and the sample codification were the following (see **Table 49**).

Table 49 - Contamination levels

Level	Samples	Theoretical target level (b/25 g)	True level (b/25 g sample)	Low limit / 25 g sample	High limit / 25 g sample
Level 0	3 – 8 – 11 – 15 – 16 – 19 – 23 – 24	0	/	/	/
Low level	1 – 5 – 7 – 9 – 13 – 17 – 20 – 22	3	4.1	3.6	4.7
High level	2 – 4 – 6 – 10 – 12 – 14 – 18 – 21	20	19.2	16.7	22.1

5.2.3 Logistic conditions

Temperature conditions are given in **Table 50** below.

Table 50 - Sample temperatures at receipt

Laboratories	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time	Date of analysis
A	7.0	11.1	Day 2 – 11h30	Day 2
B	*Not received*	16.0	Day 2 – 11h00	Day 2
C	7.5	16.0	Day 2 – 17h00	Not analyzed
D	4.5	5.2	Day 2 – 11h00	Day 2
E	7.5	12.3	Day 2 – 11h25	Day 2
G	13.0	13.9	Day 2 – 14h00	Day 2
H	4.0	3.7	Day 1 – 07h00	Day 2
I	2.5	4.2	Day 1 – 11h30	Day 1 (ISO) Day 2 (Alternative)
J	7.0	7.5	Day 1 – 13h00	Day 2
K	3.0	3.6	Day 1 – 12h00	Day 2
L	5.5	10.3	Day 2 –11h45	Day 2
M	5.0	8.0	Day 2 – 12h00	Day 2
N	7.0	8.9	Day 2 – 09h45	Day 2
O	8.4	12.0	Day 2 – 09h30	Day 2
P	7.5	11.7	Day 2 – 11h00	Day 2

Three laboratories received their samples at 8.4°C or above 8.4°C (labs B, G and O). The results of these labs were not kept for interpretation.

5.3 Results analysis

The raw data are given in **Appendix 7.**

5.3.1 Expert lab results

The results obtained by the expert laboratory are given in **Table 51**.

Table 51 – Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	8/8	8/8
L2	8/8	8/8

5.3.2 Results observed by the collaborative laboratories

- **Aerobic mesophilic flora enumeration**

Depending on the lab results, the enumeration levels varied from 6.1 x 10^2 to 1.1 x 10^4 CFU/g.

- ***Salmonella* spp. detection**

The samples were delivered to 15 labs. Due to late reception, lab C decided not to run the analyses. Data from 14 labs was received. The incubation times for the alternative method are shown in **Table 52.**

Table 52 – Enrichment incubation times

Laboratory	Incubation hours		Incubation time
	Beginning	End	
A	15h26	23h41	08h15
B	*Probe not received*		/
D	19h00	03h00	08h00
E	15h30	23h00	07h30
G	15h47	23h47	08h00
H	7h00	14h33	07h30
I	7h00	14h50	07h50
J	8h52	16h22	07h30
K	09h08	17h08	08h00
L	12h09	20h09	08h00
M	15h25	22h55	07h30
N	10h57	19h12	08h15
O	10h13	18h13	08h00
P	11h44	19h29	07h45

A total of 14 collaborators participated to the study. The results obtained are provided in **Table 53** (reference method) and **Table 54** (alternative method) below.

Table 53- Positive results by the reference method (ALL the collaborators)

Laboratory	Contamination level		
	L0	L1	L2
A	0	8	8
B	0	8	8
D	0	8	7
E	0	8	8
G	1	8	8
H	0	8	8
I	0	8	8
J	0	8	8
K	0	8	8
L	0	8	8
M	0	8	8
N	0	8	8
O	4	8	8
P	0	8	8
Total	P_0 = 5	P_1 = 112	P_2 = 111

Table 54 - Positive results (before and after confirmation) by the alternative methods (ALL the collaborators)

Laboratory	Contamination level								
	L0			L1			L2		
	PCR result	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*
A	0	0	0	8	8	8	8	8	8
B	6	0	0	8	8	8	8	8	8
D	0	0	0	8	8	8	8	8	8
E	0	0	0	8	8	8	8	8	8
G	0	0	0	8	8	8	8	8	8
H	0	0	0	8	8	8	8	8	8
I	0	0	0	8	8	8	8	8	8
J	0	0	0	8	8	8	8	8	8
K	2	0	0	8	8	8	8	8	8
L	0	0	0	8	8	8	8	8	8
M	1	0	0	8	8	8	8	8	8
N	0	0	0	8	8	8	8	8	8
O	2	2	1	8	8	8	8	8	8
P	0	0	0	8	8	8	8	8	8
Total	P0 = 11	2	CP0 = 1	P1 = 112	112	CP1 = 112	P2 = 112	112	CP2 = 112

5.3.3 Results of the collaborators retained for interpretation

According to the AFNOR technical rules, Labs with more than one positive sample at Level 0 (confirmed positive or false positive) can't be kept for interpretation. In this study, the results from Lab K (two positive PCR results not confirmed with cultural methods) were kept in order to have the required number of collaborators. This was accepted by the AFNOR technical committee during the presentation of the initial validation study (November 2013).

The results from 10 labs were finally kept for interpretation (A. D. E. H. J. K. L. M. N and P), 4 labs were not retained for the following reasons:

- Labs B, G and O: temperature at receipt above or equal to 8.4°C;
- Lab I: samples were analyzed at Day 1 for ISO method and at Day 2 for alternative method.

The results obtained for interpretation are presented in **Table 55 (**reference method) and **Table 56** (alternative method) below.

Table 55 - Positive results by the reference method (Without Labs B, G, I and O)

Laboratory	Contamination level		
	L0	L1	L2
A	0	8	8
D	0	8	7
E	0	8	8
H	0	8	8
J	0	8	8
K	0	8	8
L	0	8	8
M	0	8	8
N	0	8	8
P	0	8	8
Total	$P_0 = 0$	$P_1 = 80$	$P_2 = 79$

Table 56 - Positive results (before and after confirmation) by the alternative method (Without Labs B, G, I and O)

Laboratory	Contamination level								
	L0			L1			L2		
	PCR result	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*	*PCR result*	*Confirmation result*	*Final result*
A	0	0	0	8	8	8	8	8	8
D	0	0	0	8	8	8	8	8	8
E	0	0	0	8	8	8	8	8	8
H	0	0	0	8	8	8	8	8	8
J	0	0	0	8	8	8	8	8	8
K	2	0	0	8	8	8	8	8	8
L	0	0	0	8	8	8	8	8	8
M	1	0	0	8	8	8	8	8	8
N	0	0	0	8	8	8	8	8	8
P	0	0	0	8	8	8	8	8	8
Total	**P_0 = 3**	**0**	**CP_0 = 0**	**P_1 = 80**	**80**	**CP_1 = 80**	**P_2 = 80**	**80**	**CP_2 = 80**

5.3.4 Calculation and interpretation

- **Calculation of the specificity percentage (SP)**

The percentage specificities (SP) of the reference method and of the alternative method, using the data after confirmation, based on the results of level L0 are the following (See Table).

Table 57 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N\_}\right)\right) x\ 100\ \% =$	100.0 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N\_}\right)\right) x\ 100\ \% =$	100.0 %

N: number of all L0 tests

P_0 = total number of false-positive results obtained with the blank samples before confirmation

CP_0 = total number of false-positive results obtained with the blank samples

Calculation of the sensitivity (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 recovery was obtained only for the higher inoculation level (L2). The interpretation was done for this level.

A summary of the results of the collaborators retained for interpretation and obtained with the reference and the alternative methods for Level 2 is provided in **Table 58.**

Table 581 - Summary of the obtained results with the reference method and the alternative method for Level 2

Response	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 79**	Positive deviation (R-/A+) **PD = 1**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0 (PPND = 0)**	Negative agreement (A-/R-) **NA = 0 (PPNA = 0)**

Based on the data summarized in Table 51, 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 the following (See **59**).

Table 59 – Sensitivity, relative trueness and false positive ratio percentages

Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \; x \; 100\% =$	100.0 %
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \; x \; 100\% =$	98.8 %
Relative trueness	$RT = \frac{(PA+NA)}{N} \; x \; 100\% =$	98.8 %
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \; x \; 100\% =$	/

- **Interpretation of data**

For an **<u>unpaired study design</u>**, the difference between (ND – PD) is calculated for the level(s) where fractional recovery is obtained (so L_2 for this study). The observed value found for (ND – PD) shall not be higher than the AL. The AL is defined as $[(ND - PD)_{max}]$ and calculated per level where fractional recovery is obtained as described below using the following three parameters:

$$(p+)_{\text{ref}} = \frac{P_x}{N_x}$$

Where:

- P_x = number of samples with a positive result obtained with the reference method at level L_2 for all the collaborators
- N_x = number of samples tested at level L_2 with the reference method by all the collaborators

$$(p+)_{\text{alt}} = \frac{CP_x}{N_x}$$

Where:

- CP_x = number of samples with a confirmed positive result obtained with the alternative method at level L_2 for all the collaborators.

- N_x = number of samples tested at level L_2 with the alternative method by all the collaborators.

$$(\text{ND-PD})_{\max} = \sqrt{3N_x \times \left((p+)_{\text{ref}} + (p+)_{\text{alt}} - 2\left((p+)_{\text{ref}} \times (p+)_{\text{alt}}\right)\right)}$$

Where:

- N_x = number of samples tested for level L_2 with the reference method by all the collaborators.

The calculations are the following, according to the EN ISO 16140-2:2016 (See **Table 60**).

Table 60 - Calculations

N_X	80
$(p+)_{ref}$	98.75 %
$(p+)_{alt}$	100.00 %
AL = (ND - PD) max	1.73
ND - PD	- 1
Conclusion	**ND - PD < AL**

The EN ISO 16140-2:2016 requirements are fulfilled as (ND - PD) is lower than the AL.

- **Evaluation of the LOD 50%, LOD 95 % and 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_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017.xls The results are used only for information (see **Table 61**).

Table 61. LOD50, LOD95 and RLOD

Method	LOD 50%	LOD 95%	RLOD
Reference	0.98 (0.71, 1.35)	4.24 (3.08, 5.84)	1.00 (0.69. 1.45)
Alternative	0.98 (0.71, 1.35)	4.24 (3.08, 5.84)	

5.3.5 Inter-laboratory study conclusion

The data and interpretations comply with the EN ISO 16140-2:2016 requirements. The Thermo Scientific™ SureTect™ Salmonella species real-time PCR Assay is considered equivalent to the ISO standard.

6 CONCLUSIONS

The performances and robustness of The Thermo Scientific™ SureTect™ Salmonella species real-time PCR have been assessed during the first validation study and multiple extension and renewal studies.

A total of 7 categories were tested with PikoReal thermocyclers, and 16 categories with both 7500 Fast and QuantStudio 5 thermocyclers.

Overall, the conclusions for the Method Comparison Study are:

- The observed values for categories analyzed (ND+PPND)-PD and (ND+PPND) +PD were lower than the acceptability limit (AL) (observed values ≤ AL) for both enrichment conditions.
- The RLOD values (using the confirmed alternative method results) meet the acceptability limit for all tested categories, protocols, and thermocyclers.
- The method is specific and selective.

The inter-laboratory study conclusions are:

- The data and interpretations comply with the EN ISO 16140-2:2016 requirements.

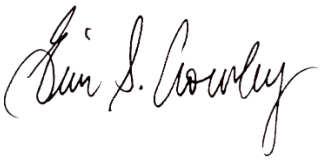
The Thermo Scientific™ SureTect™ Salmonella species real-time PCR Assay fulfils all the EN ISO 16140-2:2016 requirements and MicroVal technical rules.

Flexibility is offered with

- the sample size and enrichment protocol for multiple categories.
- the selection of the thermocyclers.
- the multiple enrichment protocols allow the end-user to have the flexibility to decide which protocol to use based on their unique needs and preferences.
- the confirmation protocol as characteristic colonies could be tested according to following options:
  - Option 1: Oxoid latex test agglutination
  - Option 2: Microbact GNB 24E biochemical galleries on isolated colonies from XLD or Brilliance Salmonella species
    - In the context of ISO general rules:
  - Option 3: Any EN ISO 16140-6:2019 validated method from isolated colonies on XLD or Brilliance Salmonella agar.
  - Option 4: ISO 6579:2017 confirmation procedure from XLD or Brilliance Salmonella agar.

On November 24, 2023,

I attest to the validation of the verification and the conformity of the report, both the opinion and interpretation. I attest to the validation of the results of the analysis carried out were under A2LA accreditation.



Erin S. Crowley

Chief Scientific Officer, Q Laboratories

APPENDIX 1: FLOW DIAGRAM OF THE ALTERNATIVE METHOD

The enrichment protocols are presented below depending on the thermocyclers and categories:

	Category or type	Enrichment step
PikoReal PCR Instrument	**General protocol with the tested categories**	
	Meat products including poultry meats Seafood and vegetables Egg products	BPW for 20 - 28 h at 34-38°C
	Infant formula (10 g) with a shorter incubation time	BPW for 16 - 24 h at 34-38°C
	Specific protocol for dairy products. due to presence of technological and background microflora	
	Dairy products	BPW + 12mg/l Novobiocin for 20-28 h at 34.38°C and ONE Broth-*Salmonella* + 12 mg/l Novobiocin for 20 - 28 h at 34-
	Short protocol for raw beef meats	
	Raw beef meats with and without aromatics	Pre-warmed BPW for 8 - 24 h at 41.5°C
	Protocol for pet food	
	Pet food	ONE Broth-*Salmonella* + ONE Broth-*Salmonella* supplement for 20 - 28 h at 34-38°C
7500 Fast and QS5 PCR Instruments	**General protocol with the tested categories**	
	Meat products Dairy products Vegetables Fish and Seafood	BPW + 12 mg/l Novobiocin for 20 - 28 h at 34-38°C
	Additional possible protocol for Dairy products	
	Dairy products	ONE Broth-*Salmonella* + 12 mg/l Novobiocin for 20 - 28 h at 34-38°C
	Infant formula	
	Infant formula (25 g)	BPW for 16 - 24 h at 34-38°C
	Milk powder. Infant formula and infant cereals with and without probiotics (from 50g to 375g) including ingredients	BPW + vancomycin (6 mg/L) for 18 - 26 h at 34-38°C
	Short protocol for raw beef meats	
	Raw beef meats with and without aromatics	Pre-warmed BPW for 9 - 24 h at 41.5°C ± 1°C
	Environmental samples	
	Environmental samples	BPW for 20 - 28 h at 34-38°C
	Cocoa and chocolate products (2 possible protocols)	
	Cocoa and chocolate products (up to 375 g)	Pre-warmed BPW for 22-30 h at 34-38°C Pre-warmed UHT milk or reconstituted non-fat dried milk (NFDM) according to the ISO 6887-4:2017 standard for 20-28 h at 34-38°C **Dilute sample 1:5 with BPW to avoid inhibition prior running the lysis step**

	Category or type	Enrichment step
7500 Fast and QS5 PCR Instruments	**Additional products for sample size up to 375 g or 150 g**	
	Pet Food (375 g)	1:6 ratio of pre-warmed (37±1°C) BPW ISO for 20-28 h at 34-38°C
	Meat Products (375 g)	1:5 ratio of pre-warmed (41.5±1°C) BPW ISO 8-24h at 41.5±1°C
	Meat Products (25 g)	1:10 ratio of pre-warmed (41.5±1°C) BPW ISO8-24h at 41.5±1°C
	Powdered Infant Formula and Cereals **with** and without **probiotics** including ingredients (e.g. caseinates) (375 g)	1:6 ratio of pre-warmed (37±1°C) BPW ISO (**suppemented with 6 mg/L novobiocin for probiotic formula only**) 18-26 h at 34-38°C
	Fresh and Processed Vegetables and Fruit (375 g)	1:10 ratio of pre-warmed (41.5±1°C) BPW ISO 10-24h at 41.5±1°C
	Animal Feed (150 g)	1:10 ratio BPW ISO with novobiocin at 12 mg/L 20-28 h at 34-38°C
	Pet Food (375 g)	1:10 ratio prewarmed (37±1°C) BPW ISO 20-28 h at 34-38°C

Perform lysis following package insert instructions (10 µL of enrichment)[3]

↓

Rehydrate SureTect PCR pellets with
(20 µL of lysate)

↓

Load and run PCR on QuantStudio5 or 7500 Fast instruments

↓

CONFIRMATION

Streaking 10µL of the enrichment broth on XLD or Brilliance Salmonella spp[4]
Confirmation tests are run from isolated colonies from XLD or Brilliance Salmonella species

- Alternative Confirmation:
  Confirmation is performed from isolated characteristic colonies from the direct streak (10 µL) of the enriched sample onto XLD or Brilliance Salmonella agars* and performing:
  - Option 1: Oxoid latex test agglutination
  - Option 2: Microbact GNB 24E biochemical galleries on isolated colonies from XLD or Brilliance Salmonella agar.
  - Option 3: Any tests described in the ISO method.

*Troubleshooting: For samples with high background microflora, a sub-culture in RVS (0.1 mL primary enrichment broth in 10 mL RVS) might be required before streaking on one of the two possible selective agars.

It is possible to store the enrichment broth at 2 to 8°C for 72h, before running the lysis step of the SureTect Salmonella spp. protocol

[1]According to the ISO 6887-4:2017standard. add Brilliant Green (0.018 g/L) for products with high background microflora
[2]According to the ISO 6887-4:2017. add Tween 80 for products containing more than 20% fat.
[3]Possible to held samples for 72 hours at 2-8°C prior DNA extraction
[4]For samples with high background microflora. a sub-culture in RVS (0.1 mL primary enrichment broth in 10 mL RVS) might be required before streaking on one of the two possible selective agars.

APPENDIX 2: FLOW DIAGRAM OF THE REFERENCE METHOD: ISO 6579-1:2017