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NF VALIDATION - Validation of alternative analysis methods
Application to the food industry

Summary report
according to the standard EN ISO 16140-2:2016

Qualitative method

Thermo Scientific™ SureTect™ Cronobacter species PCR Assay
Certificate n° UNI 03/11-12/15
for the detection of *Cronobacter* spp
in infant formula, infant cereals and related ingredients
and production environmental samples

Expert laboratory:

Laboratoire MICROSEPT
ZA de la Sablonnière
15 rue Denis Papin
49220 LE LION D'ANGERS
FRANCE

For:

Thermo Fischer Scientific
Wade Road
Basingstoke, Hampshire RG24 8PW
UNITED KINGDOM

This report contains 55 pages and 66 pages of appendices.

The accreditation of the COFRAC (Section Laboratory) gives evidence of the expertise of the laboratory for the only tests covered by the accreditation that are specified by the symbol (■).

Version 0

October 17th , 2023

Preamble

- ## Protocols of validation:

- **EN ISO 16140-1 and EN ISO 16140-2 (September 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.

- **Requirements regarding comparison and interlaboratory studies for implementation of the standard EN ISO 16140-2 (project version 7).**

- ## Reference method:

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

- ## Application scope:

- **Infant formula (10 g and 300 g),**
- **Environmental samples (25 g or surface sampling),**
- **Infant formula, infant cereals and related ingredients (up to 375 g).**

- ## Certification body:

- **AFNOR Certification** (https://nf-validation.afnor.org/).

Definitions

- ## Method comparison study

The method comparison study is the part of the validation process that is performed in the organizing laboratory. It consists of three parts namely the following:
- A comparative study of the results of the reference method to the results of the alternative method in (naturally and/or artificially) contaminated samples (so-called sensitivity study);
- A comparative study to determine the relative level of detection (RLOD) in artificially contaminated samples (so-called RLOD study);
- An inclusivity/exclusivity study of the alternative method.

  - ### Sensitivity study

The sensitivity study aims to determine the difference in sensitivity between the reference and the alternative method.
The sensitivity is the ability of the reference method or alternative method to detect the analyte.

  - ### Relative level of detection study

A comparative study is conducted to evaluate the level of detection (LOD) of the alternative method against the reference method. The evaluation is based on the calculation of the relative level of detection (RLOD).
The level of detection at 50% (LOD_{50}) is the measured analyte concentration, obtained by a given measurement procedure, for which the probability of detection is 50%.
The relative level of detection 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.

  - ### Inclusivity and exclusivity study

The inclusivity study is a study involving pure target strains to be detected or enumerated by the alternative method.
The exclusivity study is a study involving pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the alternative method.

- ## Interlaboratory study

The interlaboratory 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.
The aim of the interlaboratory study is to determine the difference in sensitivity between the reference and the alternative method when tested by different collaborators using identical samples (reproducibility conditions).

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Appendices

- **Initial study and extension studies (2016,2018 and 2021)**

- **Extension study (2023)**

1. Introduction

The SureTect Cronobacter method is validated by AFNOR Certification under the brand NF VALIDATION with the certification number UNI 03/11 – 12/15 according to the ISO 16140-2:2016 standard. The method is intended for infant formula, infant cereals and related ingredients (10 g, up to 375 g) and production environmental samples.

Table 1 summarizes the different steps of the validation that occurred since the initial validation.

Table 1: Steps of the validation AFNOR certification

Date	Study
December 2015	Initial validation study: infant formula (10 g), environmental samples - PikoReal PCR instrument
June 2016	Extension study: - Infant formula (10 g) - 7500 Fast PCR instrument - Infant formula (300 g) - PikoReal PCR instrument, and 7500 Fast PCR instrument
April 2018	Renewal study according to the ISO 22964 (2017)
December 2018	Extension study: - Infant formula (10 g) - Infant formula (up to 300 g) - Environmental samples QS5 PCR instrument
June 2021	Extension study: - Infant formula, infant cereals and related ingredients (up to 375 g)
June 2023	Extension study (Microsept): - New enrichment protocol and incubation time for infant formula, infant cereals and related ingredients (up to 375 g), and environmental samples.

This document introduces all the results of the validation studies for the NF Validation certification of the SureTect Cronobacter™ method according to the standard ISO 16140-2:2016.

2. Protocols of the methods

2.1. Alternative method

2.1.1. Principle of the method

The PCR pellets used in the SureTect assays contain lyophilized (freeze-dried) target-specific primers and dye labelled probes. Probes are short oligonucleotides with a quencher molecule at one end that greatly reduces fluorescence from the fluorophore dye at the opposite end. The oligonucleotides target unique DNA sequences found only in the target micro-organism and use PCR technology to amplify and detect them. If present, the target DNA will be amplified, and the increasing fluorescent signal detected by the PCR instrument and software running on a connected PC.

The assays used in the Thermo Scientific SureTect System are based on Solaris™ qPCR technology. The probes have a molecule called Minor Groove Binder (MGB) attached to one end, which enhances the probe-template DNA bond and yields a better signal-to-noise ratio by lowering background fluorescence. Results are achieved in around one hour of loading the prepared sample in the PCR Instrument and are displayed on the PC screen as simple positive or negative symbols with amplification plots also easily accessible for review.

The Thermo Scientific SureTect Cronobacter spp. PCR assay targets unique DNA sequences specific to all Cronobacter species. The SureTect Cronobacter species PCR Assay includes all of the necessary reagents for bacterial DNA release and PCR. Enriched samples are simply pipetted into pre-filled Lysis Tubes, along with Proteinase K, before incubation to lyse any bacterial cells present in the sample and release their DNA. The lysate is then loaded into the SureTect Cronobacter species PCR Tubes to re-hydrate the preloaded PCR pellets which contain all of the necessary components and reagents for PCR, including a probe, primers and DNA template for the internal amplification control (IAC). The PCR Tubes are then sealed, loaded into the PCR Instrument, and the run started using the software, depending on which PCR instrument is used. After running, interpreted results will be clearly displayed and can be reported, stored, printed off and downloaded as required.

The SureTect Cronobacter species PCR kit was validated with the Thermo Scientific PikoReal PCR Instrument and Thermo Scientific SureTect Software v1.2., the Applied Biosystems™ 7500 Fast with Applied Biosystems™ RapidFinder™ Express software v2.0 (or later version), and the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument using the Thermo Scientific™ RapidFinder™ Analysis Software v1.0 (or later version).

2.1.2. Protocols of the method

The workflow of the method is set out in Appendix A.

Different enrichment protocols are available depending on the categories tested. They are listed in Table 2.

Table 2: Enrichment protocols of the alternative method

	Category		Sample size	Enrichment step
Initial	PIF10	Infant formula	10 g	10 g + 90 ml BPW 16 – 24 h at 34 – 38°C
Ext. 2016 2018	ENV	Environmental samples	25 g or surface Sampling	BPW + 6 mg/l vancomycin 18 – 24h at 34 – 38°C
Ext. 2023	ENV	Environmental samples	25 g or surface Sampling	BPW 20 – 28h at 34 – 38°C
Ext. 2016 2018	PIF300	Infant formula, infant cereals and related ingredients	Up to 300 g	300 g + 2 700 ml BPW + 6 mg/l vancomycin 20 – 28h at 34 – 38°C
Ext. 2021	PIF375	Infant formula, infant cereals and related ingredients	Up to 375 g	375 g + 3 375 ml BPW 6 mg/l vancomycin 20 – 28 h at 34 – 38°C
Ext. 2023	PIF375	Infant formula, infant cereals and related ingredients	Up to 375 g	375 g + 1 875 ml pre-warmed BPW (1:6 ratio) – **Addition of novobiocin at 6 mg/L for products containing probiotics** 18 – 26 h at 34 – 38°C

The following steps of the method are identical for all categories, they are listed below:

- Lysis step on 10 µl enrichment broth,
- PCR on 20 µl lysate,
- Confirmation tests:
  - Direct streaking on CCI Agar plates, incubated for 24 h ± 2 h at 41.5°C ± 1°C;
  - Subculture in CSB (0.1 ml + 10 ml), incubated for 24 h ± 2 h at 41.5°C ± 1°C. Streaking (10 µl) onto CCI Agar plates, incubated for 24 h ± 2 h at 41.5°C ± 1°C.

  Typical colonies (blue-green) are confirmed by the tests described in the reference method.
  Note: during extension studies on 375 g, characteristic colonies were directly confirmed with biochemical galleries.

According to ISO rules, it is also appropriate to use ISO 16140-6:2019 validated confirmation methods.

In order to improve the practicability, it is possible to store the enrichment broths for 72 h at 5±3°C, except for infant formula with probiotics (10 g sample size), prior to analysis with the SureTect Cronobacter species PCR Assay.

2.1.3. Restrictions

There are no restrictions on use for the SureTect Cronobacter method.

2.2. Reference method

The current version of the reference method is the ISO 22964 standard of April 2017: Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

For the initial validation study and the previous extension studies, three versions of the standard were used:

- ISO/TS 22964 (February 2006),
- ISO/DIS 22964 (March 2015),
- ISO 22964 (April 2017).

The workflow of the reference method is presented in Appendix B and I.

2.3. Study design

For the category PIF10, the enrichment broth and test portion size being common, it is a “paired study”.

For the categories ENV, PIF300 and PIF375, tested in previous validations the enrichment broths used for the alternative and reference methods are different, the study therefore provides unpaired data, and the expression “unpaired study” is used to describe the study design.

The test samples size and incubation time tested during the extension realized in 2023 were different between the alternative method and the reference method, the design is that of an unpaired study.

In the extension realized in 2023, for the alternative method the addition of novobiocin is required for samples containing probiotics, according to the requirements of the ISO 6887 standards. The same protocol was applied to the alternative method and to the reference method.

3. Initial validation and extension studies (2016, 2018 and 2021)

3.1. Method comparison study

3.1.1. Sensitivity study

3.1.1.1. Protocols applied during the extension study

- **Incubation times:**

The broths were incubated at the minimum times of the tolerance interval for each category.

- **Confirmations:**

Presumptive positive results were confirmed by direct streaking of the enriched broth on a CCI agar plate and also after a subculture in CSB, as described in §2.1.2.

- **Cold storage of the enriched broths and Petri dishes:**

Storage of the broths for 3 days at 5±3°C was carried out, except for infant formula with probiotics (10 g sample size). The alternative method was applied from the stored enriched broths for positive and discordant samples. Only a direct streaking on CCI was performed for confirmations.

The final results are interpreted according to ISO 16140-2: 2016, using the acceptability limits of paired or unpaired methods depending on the categories.

3.1.1.2. Number and nature of the samples

The sensitivity study concerned 274 samples (cf. Table 3).

Depending on the thermal cyclers that were tested, the total number of samples tested varies from 210 to 276.

Table 3: Distribution of samples tested by category, type and thermal cycler

Category	Type		Number of samples tested with the Piko Real			Number of samples tested with the 7500 Fast			Number of samples tested with the QS5		
			Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
PIF10	a	Infant formula without probiotics	15	15	30	15	15	30	15	15	30
	b	Infant formula with probiotics	15	19	34	15	19	34	15	19	34
	Total		30	34	64	30	34	64	30	34	64
ENV	a	Process and cleaning waters	7	13	20	7	13	20	5	13	18
	b	Dusts and wastes	8	12	20	8	12	20	8	12	20
	c	Wipe, sponges, swabs	16	13	29	16	13	29	16	13	29
	Total		31	38	69	31	38	69	29	38	67
PIF300	a	Infant formula without probiotics	21	24	45	21	24	45	21	24	45
	b	Infant formula with probiotics	16	16	32	16	16	32	16	16	32
	Total		37	40	77	37	40	77	37	40	77
PIF375	a	Infant formula & Infant cereals with probiotics	/	/	/	10	12	22	10	12	22
	b	Infant formula & Infant cereals - probiotics	/	/	/	10	13	23	10	13	23
	c	Ingredients (caseinates, lecithin, etc...)	/	/	/	10	11	21	10	11	21
	Total		/	/	/	30	36	66	30	36	66
All categories			**98**	**112**	**210**	**128**	**148**	**276**	**126**	**148**	**274**

3.1.1.3. Artificial contamination

Artificial contamination was carried out using stressed strains in accordance with the requirements of the validation standard and the AFNOR Validation Technical Board (see Appendix C).

Artificial contamination was performed by:

- Spiking protocol: the strains were stressed using various injury protocols; the injury efficiency was evaluated by comparing enumeration results onto selective and non-selective agars (respectively ESIA and TSYEA),
- Seeding protocol with lyophilized strains for dehydrated products.

Table 4 gives the distribution of the positive samples per level of contamination and per thermal cycler used.

Table 4: distribution of the positive samples per contamination protocol and inoculation level (cl: contamination level in CFU/test portion)

Positive samples	Naturally contaminated samples	Artificially contaminated samples			
		Spiking	Seeding		
		cl≤5 CFU	cl ≤ 3	3 < cl ≤ 10	cl > 10
128	6	23	79	20	0
/	4.7%	18.0%	61.7%	15.6%	0%

128 samples gave a positive result by at least one of the methods and 10% of them were naturally contaminated.

3.1.1.4. Results

Raw data are shown in appendix D.

Tables 5, 6 and 7 show the results of the sensitivity study for all categories, according to the thermal cycler used.

Table 5: interpretation of the sensitivity study results (R+/-: reference method positive or negative, A+/-: alternative method positive or negative, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation) - Pikoreal

Category	Response	R+	R-
PIF10	A+	PA = 29	PD = 1
	A-	ND = 0 incl. 0 PPND	NA = 34 incl. 2 PPNA
ENV	A+	PA = 24	PD = 4
	A-	ND = 3 incl. 1 PPND	NA = 38 incl. 2 PPNA
PIF300	A+	PA = 23	PD = 9
	A-	ND = 5 incl. 0 PPND	NA = 40 incl. 3 PPNA
Total	A+	**PA = 76**	**PD = 14**
	A-	**ND = 8 incl. 1 PPND**	**NA = 112 incl.7 PPNA**

Table 6: interpretation of the sensitivity study results (R+/-: reference method positive or negative, A+/-: alternative method positive or negative, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation) – 7500 Fast

Category	Response	R+	R-
PIF10	A+	PA = 29	PD = 1
	A-	ND = 0 incl. 0 PPND	NA = 34 incl. 2 PPNA
ENV	A+	PA = 24	PD = 4
	A-	ND = 3 incl. 0 PPND	NA = 38 incl. 0 PPNA
PIF300	A+	PA = 23	PD = 9
	A-	ND = 5 incl. 0 PPND	NA = 40 incl. 1 PPNA
PIF375	A+	PA = 19	PD = 7
	A-	ND = 4 incl. 0 PPND	NA = 36 incl. 4 PPNA
Total	A+	**PA = 95**	**PD = 21**
	A-	**ND = 12 incl. 0 PPND**	**NA = 148 incl.7 PPNA**

Table 7: interpretation of the sensitivity study results (R+/-: reference method positive or negative, A+/-: alternative method positive or negative, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation) – QS5

Category	Response	R+	R-
PIF10	A+	PA = 29	PD = 1
	A-	ND = 0 incl. 0 PPND	NA = 34 incl. 2 PPNA
ENV	A+	PA = 22	PD = 4
	A-	ND = 3 incl. 0 PPND	NA = 38 incl. 1 PPNA
PIF300	A+	PA = 23	PD = 9
	A-	ND = 5 incl. 2 PPND	NA = 40 incl. 2 PPNA
PIF375	A+	PA = 19	PD = 7
	A-	ND = 4 incl. 0 PPND	NA = 36 incl. 2 PPNA
Total	A+	**PA = 93**	**PD = 21**
	A-	**ND = 12 incl. 2 PPND**	**NA = 148 incl.7 PPNA**

3.1.1.5. Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (PFR)

The set of results obtained were used to calculate the relative trueness, the sensitivity and the false positive ratio for each of the categories and for all the categories, according to the formulas set out in the EN ISO 16140-2:2016 standard.

This interpretation is shown below in Table 8 for the PikoReal thermal cycler, in Table 9 for the 7500 Fast thermal cycler and in Table 10 for the Quant Studio 5 thermal cycler.

The calculations formulae of the ISO 16140-2 standard are presented below:

Sensitivity of the alternative method:

$$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\ \%$$

Sensitivity of the alternative method:

$$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\ \%$$

Relative trueness:

$$RT = \frac{(PA + NA)}{N} \times 100\ \%$$

False positive ratio:

$$FPR = \frac{FP}{NA} \times 100\ \%$$

Table 8: values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SEalt : sensitivity for the alternative method, SEref : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method) – PikoReal thermal cycler

Category		Type	PA	NA	PD	ND	N	PPND	PPNA	SE $_{alt}$ %	SE $_{ref}$ %	RT%	FPR %
PIF10	a	Infant formula without probiotics	15	15	0	0	30	0	0	100.0%	100.0%	100.0%	0.0%
	b	Infant formula with probiotics	14	19	1	0	34	0	2	100.0%	93.3%	97.1%	10.5%
		Total	29	34	1	0	64	0	2	100,0%	96.7%	98.4%	5.9%
ENV	a	Process and cleaning waters	6	13	1	0	20	0	1	100.0%	85.7%	95.0%	7.7%
	b	Dusts and wastes	6	12	1	1	20	0	0	87.5%	87.5%	90.0%	0.0%
	c	Wipe, sponges, swabs	12	13	2	2	29	1	1	87.5%	87.5%	86.2%	15.4%
		Total	24	38	4	3	69	1	2	90,3%	87.1%	89.9%	7.9%
PIF300	a	Infant formula without probiotics	11	24	6	4	45	0	1	81.0%	71.4%	77.8%	4.2%
	b	Infant formula with probiotics	12	16	3	1	32	0	2	93.8%	81.3%	87.5%	12.5%
		Total	23	40	9	5	77	0	3	86.5%	75.7%	81.8%	7.5%
Total			**76**	**112**	**14**	**8**	**210**	**1**	**7**	91.8%	85.7%	89.5%	7.1%

Table 9: values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SEalt : sensitivity for the alternative method, SEref : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method) – 7500 Fast thermal cycler

Category		Type	PA	NA	PD	ND	N	PPND	PPNA	SE $_{alt}$ %	SE $_{ref}$ %	RT%	FPR %
PIF10	a	Infant formula without probiotics	15	15	0	0	30	0	0	100.0%	100.0%	100.0%	0.0%
	b	Infant formula with probiotics	14	19	1	0	34	0	2	100.0%	93.3%	97.1%	10.5%
		Total	29	34	1	0	64	0	2	100,0%	96.7%	98.4%	5.9%
ENV	a	Process and cleaning waters	6	13	1	0	20	0	0	100.0%	85.7%	95.0%	0.0%
	b	Dusts and wastes	6	12	1	1	20	0	0	87.5%	87.5%	90.0%	0.0%
	c	Wipe, sponges, swabs	12	13	2	2	29	0	0	87.5%	87.5%	86.2%	0.0%
		Total	24	38	4	3	69	0	0	90,3%	87.1%	89.9%	0.0%
PIF300	a	Infant formula without probiotics	11	24	6	4	45	0	0	81.0%	71.4%	77.8%	0.0%
	b	Infant formula with probiotics	12	16	3	1	32	0	1	93.8%	81.3%	87.5%	6.3%
		Total	23	40	9	5	77	0	1	86,5%	75.7%	81.8%	2.5%
PIF375	a	Infant formula & Infant cereals with probiotics	4	12	2	4	22	0	1	60.0%	80.0%	72.7%	8.3%
	b	Infant formula & Infant cereals - probiotics	7	13	3	0	23	0	0	100.0%	70.0%	87.0%	0.0%
	c	Ingredients (caseinates, lecithin, etc...)	8	11	2	0	21	0	1	100.0%	80.0%	90.5%	9.1%
		Total	19	36	7	4	66	0	2	86.7%	76.7%	83.3%	5.6%
Total			**95**	**148**	**21**	**12**	**276**	**0**	**5**	**90.6%**	**83.6%**	**88.0%**	**3.4%**

Table 10: values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SEalt : sensitivity for the alternative method, SEref : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method) – Quant Studio 5 thermal cycler

Category		Type	PA	NA	PD	ND	N	PPND	PPNA	SE $_{alt}$ %	SE $_{ref}$ %	RT%	FPR %
PIF10	a	Infant formula without probiotics	15	15	0	0	30	0	0	100.0%	100.0%	100.0%	0.0%
	b	Infant formula with probiotics	14	19	1	0	34	0	2	100.0%	93.3%	97.1%	10.5%
		Total	29	34	1	0	64	0	2	100,0%	96.7%	98.4%	5.9%
ENV	a	Process and cleaning waters	4	13	1	0	18	0	0	100.0%	80.0%	94.4%	0.0%
	b	Dusts and wastes	6	12	1	1	20	0	1	87.5%	87.5%	90.0%	8.3%
	c	Wipe, sponges, swabs	12	13	2	2	29	0	0	87.5%	87.5%	86.2%	0.0%
		Total	22	38	4	3	67	0	1	89,7%	86.2%	89.6%	2.6%
PIF300	a	Infant formula without probiotics	11	24	6	4	45	1	1	81.0%	71.4%	77.8%	8.3%
	b	Infant formula with probiotics	12	16	3	1	32	1	1	93.8%	81.3%	87.5%	12.5%
		Total	23	40	9	5	77	2	2	86,5%	75.7%	81.8%	10.0%
PIF375	a	Infant formula & Infant cereals with probiotics	4	12	2	4	22	0	1	60.0%	80.0%	72.7%	8.3%
	b	Infant formula & Infant cereals - probiotics	7	13	3	0	23	0	0	100.0%	70.0%	87.0%	0.0%
	c	Ingredients (caseinates, lecithin, etc...)	8	11	2	0	21	0	0	100.0%	80.0%	90.5%	0.0%
		Total	19	36	7	4	66	0	1	86.7%	76.7%	83.3%	2.8%
Total			**93**	**148**	**21**	**12**	**274**	**2**	**6**	**90.5%**	**83.3%**	**88.0%**	**5.4%**

The results for the category of the extension study and for all categories, per kind of thermal cycler are summarized in Table 11.

Table 11: summary of the results for all categories

Parameter	Pikoreal	7500 Fast	Quant Studio 5
	All categories	All categories	All categories
Sensitivity of the alternative method (SE_{alt})	91.8%	90.6%	90.5%
Sensitivity of the reference method (SE_{ref})	85.7%	83.6%	83.3%
Relative trueness (RT)	89.5%	88.0%	88.0%
False positive ratio (FPR)	7.1%	3.4%	5.4%

3.1.1.6. Analysis of the discordant results

Discordant results are examined according to the standard ISO 16140-2:2016.

The discordant results for Infant formula 10 g, Infant formula in 300 g and Environmental samples are presented in Tables 12 and 13.

The discordant results for Infant formula, infant cereals and related ingredients in 375 g are listed in Table 14.

Table 12: summary of the negative deviations for the categories PIF10, ENV and PIF300

Category	Type	#	Product	Artificial contamination		ISO 22964	SureTect *Cronobacter* species							Agreement		
							PCR result (Cq)			Confir-mation	Final result Piko Real	Final result 7500 Fast	Final result QS5			
				Strain	Inocu-lation level CFU/ sample		PikoReal	7500 Fast	QS5					Piko Real	7500 Fast	QS5
ENV	b	2677	Dusts	*C. sakazakii* Ad 704	2.0	**P**	-	-	-	-	**A**	**A**	**A**	**ND**	**ND**	**ND**
	c	2445	Wipe	*C. sakazakii* Ad 1428	1.0	**P**	+(42.36) / +(47.63)	-	-	-	**A**	**A**	**A**	**PPND**	**ND**	**ND**
	c	3477	Surface sponge	*C. sakazakii* Ad 893	0.2	**P**	-	-	-	-	**A**	**A**	**A**	**ND**	**ND**	**ND**
PIF 300	a	3268	Infant formula without probiotics	*C. sakazakii* Ad1446	<1.0	**P**	-	-/-	+(44.15)	-	**A**	**A**	**A**	**ND**	**ND**	**PPND**
	a	3271	Infant formula without probiotics	*C. sakazakii* Ad940	7.5	**P**	-	-	-	-	**A**	**A**	**A**	**ND**	**ND**	**ND**
	a	3759	Infant formula without probiotics	*C. malonaticus* DSM18702	3.4	**P**	-	-	-	-	**A**	**A**	**A**	**ND**	**ND**	**ND**
	a	3761	Infant formula without probiotics	*C. malonaticus* DSM18702	3.4	**P**	-	-	-	-	**A**	**A**	**A**	**ND**	**ND**	**ND**
	b	1755	Infant formula with probiotics ($8.4x10^4$ CFU/g)	*C. sakazakii* Ad916	2.8	**P**	-	-/+(19.12)	+(22.00)	-	**A**	**A**	**A**	**ND**	**ND**	**PPND**

Table 13: summary of the positive deviations for the categories PIF10, ENV and PIF300

Cat.	Type	#	Product	Artificial contamination		ISO 22964	SureTect *Cronobacter* species							Agreement		
							PCR result (Cq)			Confir-mation	Final result Piko Real	Final result 7500 Fast	Final result QS5			
				Strain	Inocu-lation level CFU/ sample		Piko Real	7500 Fast	QS5					Piko Real	7500 Fast	QS5
PIF 10	b	2306	Infant formula milk powder with probiotics (7.0x10^5/g)	*C. sakazakii* Ad 1418	4.1	**A**	+(30.12)	+(28.61)	+(29.97)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
ENV	a	2053	Rinsing water	*C.sakazakii* Ad 947	2.8	**A**	+(21.12)	+(19.58)	+(21.85)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	b	2682	Dusts	*C.sakazakii* Ad 831	1.4	**A**	+(31.71)	+(29.48)	+(30.85)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	c	2058	Wipe	*C.sakazakii* Ad 2290	1.0	**A**	+(21.54)	+(19.48)	+(21.85)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	c	2440	Wipe	*C.sakazakii* Ad 1708	0	**A**	+(31.27)	+(28.64)	+(31.29)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
PIF 300	a	3269	Infant formula without probiotics	*C.sakazakii* Ad940	7.5	**A**	+(28.06)	+(27.26)	+(30.05)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	a	3272	Infant formula without probiotics	*C.sakazakii* Ad1420	<1.0	**A**	+(32.93)	+(33.00)	+(36.12)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	a	3273	Infant formula without probiotics	*C.sakazakii* Ad1420	<1.0	**A**	+(30.72)	+(30.90)	+(33.02)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	a	3483	Infant formula without probiotics	*C.dublinensis DSM18705*	1.6	**A**	+(26.70)	+(26.70)	+(26.64)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	a	3754	Infant formula without probiotics	*C.dublinensis DSM18705*	0.8	**A**	+(30.38)	+(31.20)	+(37.52)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	a	3758	Infant formula without probiotics	*C.turicensis Ad1445*	2.0	**A**	+(35.07)	+(34.23)	+(36.73)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	b	1757	Infant formula with probiotics (2.2x10^5 cfu/g)	*C.sakazakii* Ad953	<1.0	**A**	+(28.02)	+(27.46)	+(29.80)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	b	1759	Infant formula with probiotics (1.0x10^6 cfu/g)	*C.sakazakii* Ad953	<1.0	**A**	+(25.08)	+(24.00)	+(26.71)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**
	b	3687	Infant formula with probiotics (<10 cfu/g)	/	/	**A**	+(25.22)	+(25.51)	+(27.51)	+	**P**	**P**	**P**	**PD**	**PD**	**PD**

Table 14: summary of the positive and negative deviations for the category PIF375

#	Type	Product name (probiotic enumeration CFU/g)	Contami-nation	Reference method: ISO 22964			Alternative method: SureTect Cronobacter species						Agreement	
				CCI	Confir-mation	Final result	PCR result (Cq)		Confirmation by direct streaking (aspect of the CCI)	Confirmation after subculture (aspect of the CCI)	Final result			
							7500 Fast	QS5			7500 Fast	QS5	7500 Fast	QS5
2034828	a	Infant formula milk powder with probiotics *Bifidobacterium lactis* 2.1x10^{7} CFU/g	Artificial	- (Ø)	/	**A**	+ (19.68)	+ (19.12)	+ (AM)	+ (AM)	**P**	**P**	**PD**	**PD**
2034845	a	Infant cereals with probiotics (cocoa) Batch 2 - *B. lactis* 5.6x10^{6} CFU/g	Artificial	- (Ø)	/	**A**	(+ 20.29)	+ (19.47)	+ (AM)	+ (AM)	**P**	**P**	**PD**	**PD**
2034851	b	Infant cereals w/o probiotics (vanilla)	Artificial	- (Ø)	/	**A**	+ (20.40)	+ (18.84)	+ (AM)	+ (AL)	**P**	**P**	**PD**	**PD**
2034888	b	Infant formula milk powder 6 - 12 months batch 1	Artificial	- (Ø)	/	**A**	+ (33.15)	+ (31.39)	+ (AL)	+ (AL)	**P**	**P**	**PD**	**PD**
2034853	b	Infant formula milk powder 6 - 12 months batch 2	Natural	- (Ø)	/	**A**	+ (22.87)	+ (20.33)	+ (AM)	+ (AL)	**P**	**P**	**PD**	**PD**
2034838	c	Maltodextrine batch 1	Artificial	- (Ø)	/	**A**	+ (20.38)	+ (19.44)	+ (AM)	+ (AM)	**P**	**P**	**PD**	**PD**
2055441	c	Rice flour batch 2	Natural	+ (EM)	/	**A**	+ (24.30)	+ (23.25)	+ (BM)	+ (BM)	**P**	**P**	**PD**	**PD**
2034824	a	Infant formula milk powder with probiotics *Lactobacillus reuteri* 5.5x10^{6} CFU/g	Artificial	+ (AM)	-	**P**	-	-	- (Ø)	- (Ø)	**A**	**A**	**ND**	**ND**
2034827	a	Infant formula milk powder with probiotics *Bifidobacterium lactis* 4.5x10^{6} CFU/g	Artificial	+ (AM)	-	**P**	-	-	- (Ø)	- (Ø)	**A**	**A**	**ND**	**ND**
2034892	a	Infant cereals with probiotics *B. lactis* 6.8x10^{6} CFU/g	Artificial	+ (AL)	-	**P**	-	-	- (EL)	- (EL)	**A**	**A**	**ND**	**ND**
2066812	a	Infant cereals with probiotics (chocolate & biscuit) - *B. lactis* 7.0x10^{5} CFU/g	Artificial	+ (AM)	-	**P**	-	-	- (EL)	- (Ø)	**A**	**A**	**ND**	**ND**

Overall, twenty-one positive deviations were observed: three from naturally contaminated samples and eighteen from artificially contaminated samples.

Twelve negative deviations were also observed: all from artificially contaminated samples. The presence of *Cronobacter* in the alternative method broth was not detected, even using the additional confirmation protocol of the ISO 16140-2 standard (subculture in CSB before streaking on CCI).

In conclusion, the negative deviations and the positive deviations most probably come from the nature of the study design. In an unpaired study, because of the difference of sampling between the two methods, and the use of naturally contaminated samples or seeded samples with low levels of contamination, no cell of *Cronobacter* may have been present in the sampling of one of the two methods.

3.1.1.7. Confirmations

No differences were observed between the two protocols used for the confirmations (direct streaking on CCI or after a subculture in CSB) during this extension study.
Three presumed positive not confirmed results were obtained during the extension study: two with the 7500 Fast thermal cycler and one with the QS5 thermal cycler.

No negative result with the SureTect method has been confirmed after a subculture in CSB.

3.1.1.8. Calculation and interpretation of data

The following Tables shows the difference between negative deviations and positive deviations and the acceptability limits according to the kind of thermal cycler used:

- Table 15: PikoReal,
- Table 16: 7500 Fast,
- Table 17: Quant Studio 5.

Table 15: acceptability limits for the PikoReal thermal cycler

Category	Types	ND	PD	Paired protocol				Unpaired protocol		Combined	
				ND-PD	AL	ND+PD	AL	ND-PD	AL	ND-PD	AL
PIF10	a	0	0	0	/	0	/	/		0	/
	b	0	1	-1		1				-1	
	Total	0	1	-1	3	1	6			-1	3
ENV	a	0	1	/				-1	/	-1	/
	b	1	1					0		0	
	c	2	2					0		0	
	Total	3	4					-1	3	-1	3
PIF300	a	4	6					-2	/	-2	/
	b	1	3					-2		-2	
	Total	5	9					-4	3	-4	3
All categories		**8**	**14**	**/**	**/**	**/**	**/**	**/**	**/**	-6	**5**

Table 16: acceptability limits for the 7500 Fast thermal cycler

Category	Type	ND	PD	Paired protocol				Unpaired protocol		Combined	
				ND-PD	AL	ND+PD	AL	ND-PD	AL	ND-PD	AL
PIF10	a	0	0	0	/	0	/	/		0	/
	b	0	1	-1		1				-1	
	Total	0	1	-1	3	1	6			-1	3
ENV	a	0	1	/				-1	/	-1	/
	b	1	1					0		0	
	c	2	2					0		0	
	Total	3	4					-1	3	-1	3
PIF300	a	4	6					-2	/	-2	/
	b	1	3					-2		-2	
	Total	5	9					-4	3	-4	3
PIF375	**a**	**4**	**2**					**2**		**2**	
	b	**0**	**3**					**-3**		**-3**	
	c	**0**	**2**					**-2**		**-2**	
	Total	**4**	**7**					**-3**	**3**	**-3**	**3**
All categories		**12**	**21**	**/**	**/**	**/**	**/**	**/**	**/**	**-9**	**5**

Table 17: acceptability limits for the QS5 thermal cycler

Category	Type	ND	PD	Paired protocol				Unpaired protocol		Combined	
				ND-PD	AL	ND+PD	AL	ND-PD	AL	ND-PD	AL
PIF10	a	0	0	0	/	0	/	/		0	/
	b	0	1	-1		1				-1	
	Total	0	1	-1	3	1	6			-1	3
ENV	a	0	1	/				-1	/	-1	/
	b	1	1					0		0	
	c	2	2					0		0	
	Total	3	4					-1	3	-1	3
PIF300	a	4	6					-2	/	-2	/
	b	1	3					-2		-2	
	Total	5	9					-4	3	-4	3
PIF375	**a**	**4**	**2**					**2**		**2**	
	b	**0**	**3**					**-3**		**-3**	
	c	**0**	**2**					**-2**		**-2**	
	Total	**4**	**7**					**-3**	**3**	**-3**	**3**
All categories		**12**	**21**	**/**	**/**	**/**	**/**	**/**	**/**	**-9**	**5**

The observed values (ND – PD) are below the acceptability limits for each category and for all categories for the three kinds of thermal cyclers tested. The alternative method produces results comparable to the reference method.

3.1.1.9. Enrichment broth storage at 2 – 8°C for 72 hours

A stability study of the enriched broths stored at 5±3°C for 72 hours was performed on positive and discordant samples. After storage, the broths were reanalyzed and confirmed. The test portions of 10 g of infant formula powders (PIF10, type b) with probiotics were not covered by this study.

During the initial validation study, the conclusion changed for two samples analyzed with PikoReal and with 7500 Fast thermal cyclers and for three samples analyzed with Quant Studio 5 thermal cycler.
These samples were all belonging to the type a of the PIF300 category: Infant formula without probiotic.
They all moved from NA to PD (cf. summay report).

During the current extension study, the conclusion for only one sample changed after storage at 2-8°C for 3 days: sample 2034852, Infant cereals w/o probiotics (wheat & cocoa), which was negative just after enrichment, and became positive after the cold storage.
The concordance between the reference and the alternative method moved from NA to PD.

The interpretation of the results after cold storage is presented in the following Tables according to the kind of thermal cycler used:

- Table 18: PikoReal,
- Table 19: 7500 Fast,
- Table 20: Quant Studio 5.

Table 18: acceptability limits for the PikoReal thermal cycler after cold storage of the broths

Category	Types	ND	PD	Paired protocol				Unpaired protocol		Combined	
				ND-PD	AL	ND+PD	AL	ND-PD	AL	ND-PD	AL
PIF10	a	0	0	0	/	0	/	/		0	/
	Total	0	0	0	3	0	6			0	3
ENV	a	0	1	/				-1	/	-1	/
	b	1	1					0		0	
	c	2	2					0		0	
	Total	3	4					-1	3	-1	3
PIF300	a	4	8					-4	/	-4	/
	b	1	3					-2		-2	
	Total	5	11					-6	3	-6	3
All categories		**8**	**15**	**/**	**/**	**/**	**/**	**/**	**/**	-7	**5**

Table 19: acceptability limits for the 7500 Fast thermal cycler after cold storage of the broths

Category	Type	ND	PD	Paired protocol				Unpaired protocol		Combined	
				ND-PD	AL	ND+PD	AL	ND-PD	AL	ND-PD	AL
PIF10	a	0	0	0	/	0	/	/		0	/
	Total	0	0	0	3	0	6			0	3
ENV	a	0	1	/				-1	/	-1	/
	b	1	1					0		0	
	c	2	2					0		0	
	Total	3	4					-1	3	-1	3
PIF300	a	4	8					-4	/	-4	/
	b	1	3					-2		-2	
	Total	5	11					-6	3	-6	3
PIF375	**a**	**4**	**2**					**2**		**2**	
	b	**0**	**4**					**-4**		**-4**	
	c	**0**	**2**					**-2**		**-2**	
	Total	**4**	**8**					**-4**	**3**	**-4**	**3**
All categories		**12**	**23**	**/**	**/**	**/**	**/**	**/**	**/**	**-11**	**5**

Table 20: acceptability limits for the QS5 thermal cycler after cold storage of the broths

Category	Type	ND	PD	Paired protocol				Unpaired protocol		Combined	
				ND-PD	AL	ND+PD	AL	ND-PD	AL	ND-PD	AL
PIF10	a	0	0	0	/	0	/	/		0	/
	Total	0	0	0	3	0	6			0	3
ENV	a	0	1	/				-1	/	-1	/
	b	1	1					0		0	
	c	2	2					0		0	
	Total	3	4					-1	3	-1	3
PIF300	a	4	9					-5	/	-5	/
	b	1	3					-2		-2	
	Total	5	12					-7	3	-7	3
PIF375	**a**	**4**	**2**					**2**		**2**	
	b	**0**	**4**					**-4**		**-4**	
	c	**0**	**2**					**-2**		**-2**	
	Total	**4**	**8**					**-4**	**3**	**-4**	**3**
All categories		**12**	**24**	**/**	**/**	**/**	**/**	**/**	**/**	**-12**	**5**

After cold storage of the enriched broths, the observed values (ND – PD) are below the acceptability limits for each category and for all categories for the three kinds of thermal cyclers tested.

The alternative method produces results comparable to the reference method.

3.1.1.10. PCR inhibitions

Some inhibitions were observed during the initial validation study.
No inhibition was observed during the extension study (PIF375).

All samples on which inhibitions were observed are listed in Table 21, as well as the percentage of inhibition for each thermal cycler used with regard to the number of samples analyzed.

Table 21: PCR inhibitions (: 1/5 dilution,**: Discordant result between 7500 Fast - initial validation result - and QS5, lysates tested again with 7500 Fast in 2018)*

Sample n°	Product	PCR result		
		PikoReal	7500 Fast	QS5
2310	Infant formula milk powder with probiotics ($3.6x10^6$ CFU/g) (10 g)	i/-*	-	-
3947	Infant formula without probiotics (300 g)	i/-* (after storage)	-/+ (37.42)**	+ 38.35
1469	Infant formula milk powder with probiotics ($1.2x10^7$ CFU/g)	-	i/-*	-
3264	Infant formula with probiotics ($3.9x10^5$ CFU/g)	+ (19.70)	i/+ (20.24)*	+ (21.92)
2304	Infant formula (10 g) with probiotics ($5.4x10^6$/g)	+ (30.57)	+ 27.53	i/+ (28.41)*
2052	Rinsing water	+ (21.15)	+ (20.16)	i/ + (24.97)*
4078	Rinsing water	-	-	i/-*
3372	Infant formula without probiotics	-	-	i/-*
% of inhibition		**0,9**	**0,7**	**1,4**

A 1/5th dilution was applied: 3 samples gave then a positive result and 5 samples a negative result.

The percentage of inhibition varies for 0.6 to 1.3 % depending on the thermocycles used.

3.1.2. Relative level of detection study

3.1.2.1. Matrix-strain pairs

Table 22 summarizes the matrix-strain pairs tested and the kind of thermal cyclers on which it was analyzed.

Table 22: matrix-strain pairs for the determination of the RLOD

Category	Matrix	Inoculated strain	Storage conditions before analysis	Thermal cycler tested
Infant formula (10 g sample size) PIF10	Infant formula without probiotics	*Cronobacter sakazakii* Ad1418	2 weeks at room temperature	PikoReal 7500 Fast
	Infant formula with probiotics	*Cronobacter sakazakii* Ad1418	2 weeks at room temperature	
Production environmental samples ENV	Process water	*Cronobacter turicensis* Ad1445	4°C, 48 - 72 h	PikoReal 7500 Fast
Infant formula (up to 300 g sample size) PIF300	Infant formula with probiotics	*Cronobacter sakazakii* Ad1446	2 weeks at room temperature	PikoReal 7500 Fast QS5
Infant formula, infant cereals and related ingredients (up to 375 g sample size) PIF375	Infant cereals with probiotics	*Cronobacter dublinensis* GVV828, wild strain isolated from a milk powder	Lyophilized strain with stabilization after inoculation for 2 weeks at room temperature	7500 Fast QS5

3.1.2.2. Contamination protocol

Three levels of contamination were prepared consisting of a negative control level, a low level, and a higher level.
The negative control level shall not produce positive results. Five replicates were tested for this level.
The low level shall be the theoretical detection level, it was contaminated at 0.7 - 1 CFU per test portion to obtain fractional recovery results. Twenty replicates were tested for this level.
The higher level shall be just above the theoretical detection level, it was contaminated at 2 - 3 CFU per test portion. Five replicates were tested for this level.

3.1.2.3. Results

The detailed results table is set out in Appendix E for the category PIF375 tested during the extension study. Data from the previous validation studies are available in the summary report of the method.

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method: RLOD= LOD_{alt} / LOD_{ref}.
The RLODs calculations were performed according to the standard ISO 16140-2: 2016 using the Excel spreadsheet available for download at http://standards.iso.org/iso/16140, with unknown

concentrations.Values of the RLODs are set out in Tables 23, 24 and 25 according to the king of thermal cycler used for the tests.

Table 23: RLODs values for all categories with the PikoReal thermal cycler (RLOD: the estimated relative level of detection value, RLODU: the upper limit of the 95% confidence interval for RLOD, RLODL: the lower limit of the 95% confidence interval for RLOD)

Name	RLOD	RLODL	RLODU	AL
Infant formula (10g) with probiotics/C.sakazakii Ad1418	1.148	0.485	2.713	1.5
Infant formula (10g) without probiotics/C.sakazakii Ad1418	1.000	0.478	2.092	1.5
Process water/C.turicensis Ad1445	1.038	0.449	2.399	2.5
Infant formula (300g) with probiotics/C.sakazakii Ad1446	1.482	0.704	3.118	2.5
Combined	**1.166**	**0.801**	**1.700**	**/**

Table 24: RLODs values for all categories with the 7500 Fast thermal cycler(RLOD: the estimated relative level of detection value, RLODU: the upper limit of the 95% confidence interval for RLOD, RLODL: the lower limit of the 95% confidence interval for RLOD)

Name	RLOD	RLODL	RLODU	AL
Infant formula (10g) with probiotics / *C.sakazakii* Ad1418	1.000	0.457	2.187	1.5
Infant formula (10g) without probiotics / *C.sakazakii* Ad1418	1.000	0.478	2.092	1.5
Process water / *C.turicensis* Ad1445	1.038	0.449	2.399	2.5
Infant formula (300g) with probiotics / *C.sakazakii* Ad1446	1.482	0.704	3.118	2.5
Infant cereals (375 g) with probiotics / *C. dublinensis* GVV828	1.146	0.498	2.636	2.5
Combined	**1.140**	**0.814**	**1.595**	**/**

Table 25: RLODs values for all categories with the Quant Studio 5 thermal cycler (RLOD: the estimated relative level of detection value, RLODU: the upper limit of the 95% confidence interval for RLOD, RLODL: the lower limit of the 95% confidence interval for RLOD)

Name	RLOD	RLODL	RLODU	AL
Infant formula (300g) with probiotics / *C.sakazakii* Ad1446	1.482	0.704	3.118	2.5
Infant cereals (375 g) with probiotics / *C. dublinensis* GVV828	1.146	0.498	2.636	2.5
Combined	**1.342**	**0.789**	**2.281**	**/**

3.1.2.4. Interpretation and conclusion

The RLODs values are below the acceptability limit set at 2.5 for “unpaired” categories and 1.5 for “paired” categories, meaning that, as stated in ISO 16140-2:2016, the maximum increase in LOD of the alternative versus the reference method is not considered as relevant in consideration of the fitness for purpose of the method.

In conclusion, alternative and reference methods show similar LODs values for the detection of *Cronobacter* spp in the categories tested.

3.1.2.5. Expression of the LOD values

The LOD_{50} calculations according to Wilrich & Wilrich POD-LOD calculation program - version 11, are given in Table 26.

Table 26: LOD50% for the alternative and reference method in CFU/test portion

Categories	Reference method ISO 22964	Alternative method		
		PikoReal	7500 Fast	Quant Studio 5
PIF10 with probiotics	2.735	3.053	2.735	/
PIF10 without probiotics	1.677	1.677	1.677	/
ENV	0.911	0.959	0.959	/
PIF300	0.902	1.379	1.379	1.379
PIF375	1.254	/	1.410	1.410

3.1.3. Inclusivity and exclusivity study

Inclusivity and exclusivity were tested only on PikoReal PCR Instrument during the initial validation study.
The inclusivity and exclusivity studies were not performed on 7500 Fast and QS5 Instrument during the extension studies in 2016, 2018 and 2021 with the agreement of the Technical Board.

3.1.3.1. Test protocols

- **Inclusivity**

Fifty-seven *Cronobacter* spp. isolates were cultured in BHI Broth at 37°C. Dilutions were done in order to inoculate 10 to 100 cells/225 ml of BPW + vancomycin (6 mg/l). The enrichment broth was then incubated for 18 h at 37°C and the alternative method protocol was applied.

- **Exclusivity**

Negative strain cultures were performed in BHI Broth at 37°C. Dilutions were realised in order to inoculate 10^5 cells/ml of BPW. The alternative protocol was then performed after an enrichment time of 24 hours at 37°C.

3.1.3.2. Results

Raw data are given in Appendix F.

- **Inclusivity**

All 57 *Cronobacter* spp. isolates gave a positive PCR result and positive confirmation tests using the PikoReal instrument.

- **Exclusivity**

All the 31 negative strain cultures gave a negative PCR result using the PikoReal.

3.1.3.3. Conclusion

The alternative method is specific and selective.

3.1.4. Practicability

The alternative method practicability was evaluated according to the AFNOR criteria relative to method comparison study.

- **Storage conditions of the kit**

The reagents remain stable until the expiry date stated on the packaging.
The complete box and contents should be stored at 2 - 8 °C when not in use.

- **Storage conditions after first utilization**

Do not use the reagent after the expiration date indicated on the label.
For SureTect PCR strips, unused materials must be returned to the refrigerator after a short time.

- **Time-to-result**

See table 27 below.

Table 27: time-to-result of the alternative method, compared to the reference method

Steps	Reference method	Alternative method
Negative samples		
Sampling	Day 0	Day 0
Extraction / PCR	/	Day 1
Subculture	Day 3	/
Final negative result	Day 3	Day 1
Presumptive samples or positive samples		
Confirmation	Day 5	Day 2 or Day 3

- **Common step with the reference method**

The enrichment step is common with the reference method only for the infant formula.

3.2. Interlaboratory study

3.2.1. Study organization

Samples were sent to 15 laboratories. This study was done with probiotic infant formula inoculated with *Cronobacter sakazakii* Ad 940 strain. In order to facilitate the study, infant formula was first homogenized in sterile water.

Samples were inoculated on Monday 19 October 2015; as described below:

- 24 blind coded samples (10 ml) for *Cronobacter* spp. detection by the Thermo Scientific™ SureTect™ Cronobacter species PCR Assay and the ISO/TS 22964 (2006) reference method;
- 1 sample (labelled "Sample for anaerobic lactic flora") for anaerobic lactic flora enumeration in MRS agar,
- 1 water flask labelled "Temperature Control" with a temperature probe for temperature control during transport and storage in the laboratory until the beginning of the analysis.

The targeted inoculation levels were the following:

- Level 0: 0 CFU/g,
- Level 1: 1 CFU/10 g,
- Level 2: 10 CFU/10 g.

Each laboratory received 24 samples of 10 g, i.e. 8 samples per inoculation level and method. Furthermore, one non-inoculated sample was added to the package for lactic microflora enumeration.

Blind coded 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 analysis.

Samples were shipped within 24 h to each of the participating laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C – 8°C on arrival at each of the labs.

Collaborators and the expert laboratory carried out the analysis at Day 1 or Day 2 with the alternative and reference methods.

In order to evaluate the *Cronobacter* spp. strain stability during transport, *Cronobacter* spp. enumerations were performed at different times, i.e. inoculation time, after 24 h and 48 h of storage at 2°C.

3.2.2. Experimental control parameters

3.2.2.1. Strain stability and background microflora stability

In order to detect *Cronobacter* spp., the reference method was performed on nine portions (10 g) before the inoculation. All the results were negative.

Three samples inoculated at a high level were tested for enumeration after 24 h and 48 h storage. Three samples inoculated at a low level were tested for detection after 48 h storage (See Table 28).

Table 28: Cronobacter sakazakii stability in the matrix

Day of analysis	***Cronobacter sakazakii* enumeration**		**Lactic flora enumeration (cfu/g)**
	ESIA (CFU/g)	ISO/TS 22964	
Day 0	1 100	+	100 000
	1 200	-	
	1 200	+	
Day 1	1 200	/	/
	1 000	/	
	890	/	
Day 2	640	+	57 000
	750	+	
	760	+	

The enumeration of *Cronobacter sakazakii* decreases slightly after storage for 48 h at 2 - 8°C.

3.2.2.2. Contamination levels

The contamination levels and the sample codifications were the following (See Table 29).

Table 29: Contamination levels and sample codifications

Level	**Samples**	**Theoretical target level**	**True level** (CFU/10 g sample)	**Low limit / CFU/10 g sample**	**High limit / CFU/10 g sample**
Level 0	2 - 6 - 8 - 11 - 15 - 19 - 22 - 23	0	/	/	/
Low level	3 - 7 - 9 - 13 16 - 18 21 - 24	1	**1.0**	0.8	1.3
High level	1 - 4 - 5 - 10 - 12 - 14 17 - 20	10	**8.6**	7.0	10.7

3.2.2.3. Logistic conditions

The temperatures measured on reception by the labs and those registered by the thermo-probes, along with the receipt dates are given in Table 30 below.

All the samples were delivered in appropriate conditions. Temperatures during shipment and at receipt were all correct. Two labs measured a temperature at receipt above 8.4°C, but the curves show clearly that the samples arrived at the expected temperature. Some leaks were mentioned for 5 Labs (A, F, H, I and J).

Table 30: Sample temperatures at receipt

Collaborator	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time		Analysis date
A	3.5	5.0	20/10/2015	14h00	20/10/2015
B	6.5	7.2	21/10/2015	13h55	21/10/2015
C	3.0	4.2	20/10/2015	12h15	/
D	Probe discarded	4.0	20/10/2015	11h00	20/10/2015
E	3.5	3.4	20/10/2015	11h05	20/10/2015
F	3.0	3.5	20/10/2015	09h45	20/10/2015
G	Not received yet	**11.2**	21/10/2015		21/10/2015
H	3.5	7.4	20/10/2015	11h45	20/10/2015
I	3.5	**8.8**	20/10/2015	13h41	20/10/2015
J	2.0	6.0	20/10/2015	12h10	21/10/2015
K	4.0	8.1	20/10/2015	11h15	20/10/2015
L	3.5	4.5	20/10/2015	11h10	20/10/2015
M	3.0	4.6	20/10/2015	11h15	/
N	3.5	6.2	20/10/2015	14h30	/
O	3.0	4.1	20/10/2015	12h00	21/10/2015

3.2.3. Summary of the results

The raw data are given in Appendix G.

3.2.3.1. Expert laboratory results

The results obtained by the Expert Lab are given in Table 31.

Table 31: Results obtained by the expert Lab

Level	Reference method	Alternative method
L_0	0/8	0/8
L_1	2/8	2/8
L_2	8/8	8/8

3.2.3.2. Collaborators results

- **Aerobic mesophilic flora**

The enumeration of the aerobic mesophilic flora varied from 1.9 x 10^4 to 1.5 x 10^5 CFU/g.

- ***Cronobacter* spp detection**

Fifteen collaborators participated to the study. The results obtained are provided in Table 32 for reference and alternative methods.

Table 32: Positive results of reference and alternative methods (P_x: number of positive results for the reference method and number of presumed positive results for the alternative method, CP_x: number of confirmed positive results for the alternative method)

Lab	Contamination level											
	Reference method results			Alternative method results								
	L_0	L_1	L_2	L_0			L_1			L_2		
				PCR	Conf.	Final	PCR	Conf.	Final	PCR	Conf.	Final
A	0	7	8	0	0	0	7	7	7	8	8	8
B	0	5	8	0	0	0	5	5	5	8	8	8
C	0	4	8	0	0	0	4	4	4	8	8	8
D	2	6	8	2	2	0	6	5	5	8	8	8
E	0	5	8	1	0	0	5	5	5	8	8	8
F	0	5	8	0	0	0	5	5	5	8	8	8
G	1	4	8	0	0	0	5	5	5	8	8	8
H	0	4	8	0	0	0	4	4	4	8	8	8
I	0	3	8	0	0	0	3	3	3	8	8	8
J	0	5	8	0	0	0	5	5	5	8	8	8
K	0	6	8	2	0	0	7	6	6	8	8	8
L	0	5	8	3	0	0	6	6	6	8	8	8
M	0	5	8	0	0	0	6	6	6	8	8	8
N	0	5	8	2	0	0	4	4	4	8	8	8
O	0	6	8	0	0	0	6	6	6	8	8	8
Total	P_0 3	P_1 75	P_2 120	P_0 10	2	CP_0 0	P_1 78	76	CP_1 76	P_2 120	120	CP_2 120

With the reference method, two labs found positive results with unspiked samples:

- Lab D: D22 – D23
- Lab G: G15

With the alternative method, five labs found positive results with unspiked samples:

- Lab D: D8 – D23
- Lab E: E19
- Lab K: K15 – K23
- Lab L: L15 – L19 – L23
- Lab N: N2 – N8

None of these ten samples were confirmed as positive with cultural procedures. Note that lab L repeated the PCR tests and got negative results.

According to the AFNOR technical rules, data from labs with more than one contamination at Level 0 were removed for interpretation, i.e. Lab D, Lab K, Lab L and Lab N.

Microbact™ galleries were provided to run the characteristic colony confirmation. Some labs encountered difficulties in the gallery's interpretation. Despite the identifications not fitting with the expected results, their results were taken into account based on the recovery of characteristic colonies. Note that the characteristic colonies were identified to *Cronobacter* spp. by the labs familiar with the use of the Microbact™ gallery.

In particular event of discordant results with the biochemical identification kit (difficulty for reading), the labs should refer to the technical instructions.

Finally, the results from 11 labs were kept: A, B, C, E, F, G, H, I, J, M and O.

3.2.3.3. Collaborators results retained for the interpretation

The results obtained with the 11 labs kept for interpretation are presented in Table 33.

Table 33: Positive results of reference and alternative methods kept for interpretation (P_x: number of positive results for the reference method and number of presumed positive results for the alternative method, CP_x: number of confirmed positive results for the alternative method)

Lab	Contamination level											
	Reference method results			Alternative method results								
				L_0			L_1			L_2		
	L_0	L_1	L_2	PCR	Conf.	Final	PCR	Conf.	Final	PCR	Conf.	Final
A	0	7	8	0	0	0	7	7	7	8	8	8
B	0	5	8	0	0	0	5	5	5	8	8	8
C	0	4	8	0	0	0	4	4	4	8	8	8
E	0	5	8	1	0	0	5	5	5	8	8	8
F	0	5	8	0	0	0	5	5	5	8	8	8
G	1	4	8	0	0	0	5	5	5	8	8	8
H	0	4	8	0	0	0	4	4	4	8	8	8
I	0	3	8	0	0	0	3	3	3	8	8	8
J	0	5	8	0	0	0	5	5	5	8	8	8
M	0	5	8	0	0	0	6	6	6	8	8	8
O	0	6	8	0	0	0	6	6	6	8	8	8
Total	P_0 1	P_1 53	P_2 88	P_0 1	0	CP_0 0	P_1 55	55	CP_1 55	P_2 88	88	CP_2 88

3.2.4. Calculation and interpretation of the results

3.2.4.1. 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 34).

Table 34: Specificity percentages (N: number of all L_0 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)

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N\_}\right)\right) x\ 100\ \% =$	98.9%
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N\_}\right)\right) x\ 100\ \% =$	100%

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

Fractional positive results were obtained for the low inoculation level (L_1). The interpretation was thus done for this inoculation level.
A summary of the results of the collaborators retained for interpretation and obtained with the reference and the alternative methods for Level 1 is provided in Table 35.

Table 35: Summary of the obtained results with the reference method and the alternative method for Level 1

Response	Reference method positive (R+)	Reference method negative (R-)
Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 54**	Positive deviation (R-/A+) **PD = 1**
Alternative method negative (A-)	Negative deviation (A-/R+) **ND = 0**	Negative agreement (A-/R-) **NA = 33**

Based on the data summarized in Table 33, 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 Table 36).

Table 36: 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:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} x\ 100\% =$	98.2%
Relative trueness	$RT = \frac{(PA+NA)}{N} x\ 100\% =$	98.9%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} x\ 100\% =$	0.0%

3.2.4.3. Interpretation of the data

For a paired study design, the difference between (ND – PD) and the addition (ND + PD) are calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (ND – PD) and (ND + PD) shall not be higher than the AL.

For 11 Labs, the calculated values and the acceptability limits are the following (See Table 37).

Table 37: acceptability limits

Values	Calculated values	AL	Conclusion
ND - PD	-1	4	ND - PD ≤ AL
ND + PD	1	4	ND + PD ≤ AL

The EN ISO 16140-2:2016 requirements are fulfilled as (ND - PD) and (ND + PD) meet the AL (calculated values ≤ AL).

3.2.4.4. Evaluation of the RLOD between laboratories

The RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at http://standards.iso.org/iso/16140/-2/ed-1/en/RLOD_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017.xls. The results are used only for information (see Table 38).

Table 38: acceptability limits

Method	LOD50%	LOD95%	RLOD
Reference	0.73 [0.54 ; 0.97]	3.15 [2.35 ; 4.21]	0.97 [0.69-1.36]
Alternative	0.70 [0.53 ; 0.94]	3.04 [2.28 ; 4.07]	

3.3. Conclusion of the initial validation and extensions (2016,2018 and 2021)

The comparative study of the methods was performed according to the EN ISO 16140-2:2016 standard.
Tests were performed with 3 thermal cyclers (depending on the categories tested): PikoReal, 7500 Fast and Quant Studio 5 PCR instruments.

- **Sensitivity study**

The performance of the SureTect Cronobacter method was compared to that of the ISO 22964 reference method by analyzing 66 samples.
The observed values (ND – PD) were below the acceptability limits for each category and for all categories after the initial test and after three days of conservation at 5±3°C.
Statistically, the alternative method produces results comparable to that of the reference method.

- **Relative level of detection study**

The relative detection level of the SureTect Cronobacter method and reference method was evaluated by artificially contaminating matrices of the application scope.
The SureTect Cronobacter method and the reference method showed similar LODs values for the detection of *Cronobacter* spp in the categories tested.

- **Inclusivity and exclusivity study**

The specificity of the method is satisfactory, as all target strains were detected (inclusivity) and no cross-reactions were observed among non-targeted tested strains (exclusivity).

- **Interlaboratory study**

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

The Thermo Scientific™ SureTect™ Cronobacter species PCR Assay is considered equivalent to the ISO 22964:2017 method.
SureTect™ Cronobacter species PCR Assay is a valuable method for the testing of infant formula with and without probiotics, infant cereals, ingredients linked to the preparation of infant formula and the testing of environmental samples.

4. Extension study realized in 2023

4.1. Sensitivity study

The study was conducted on a variety of samples and strains representative of food products. This is not an exhaustive list of the various matrices included in the application scope. For any remark on the alternative method, you can contact AFNOR Certification by connecting to the Internet page ***http://nf-validation.afnor.org/contact-2/***.

4.1.1. Protocols

- **Incubation times:**

The broths were incubated at the minimum times of the tolerance interval for each category.

- **Confirmations:**

Presumptive positive results were confirmed by direct streaking of the enriched broth on a *Brilliance* CCI agar plate and also after a subculture in CSB, as described in §2.1.2.

- **Cold storage of the enriched broths:**

Storage of the broths for 3 days at 5±3°C was carried out. The alternative method was applied from the stored enriched broths for positive and discordant samples. A direct streaking on CCI was performed following by a confirmation with micro-gallery. The final results are interpreted according to ISO 16140-2: 2016, using the acceptability limits of unpaired methods for the two categories tested.
The workflow of the method is set out in Appendix H

4.1.2. Number and nature of the samples

The sensitivity study of the extension concerns 138 samples.
During this extension, the tests were carried out using the 7500 Fast and QS5 thermal cyclers.
Results are presented in table 39.

Table 39: Distribution of samples tested by category, type and thermal cycler

	Cat.	Type		Number of samples tested with the 7500 Fast			Number of samples tested with the QS5		
				Positive	Negative	Total	Positive	Negative	Total
Extension study	PIF375	a	Infant formula & Infant cereals with probiotics	11	10	21	11	10	21
		b	Infant formula & Infant cereals w/o probiotics	9	15	24	9	15	24
		c	Ingredients (caseinates, lecithin, etc...)	10	10	20	10	10	20
		Total		30	35	65	30	35	65
	ENV	a	Dusts and Residues	8	12	20	8	12	20
		b	Cleaning and Process Waters	11	15	26	11	15	26
		c	Surface samples	12	15	27	12	15	27
		Total		31	42	73	31	42	73
	All categories			**61**	**77**	**138**	**61**	**77**	**138**

4.1.3. Artificial contamination

Artificial contamination was carried out using stressed strains in accordance with the requirements of the validation standard and the AFNOR Validation Technical Board (see Appendix J).

Artificial contamination was performed by:

- Spiking protocol: the strains were stressed using various injury protocols; the injury efficiency was evaluated by comparing enumeration results onto selective and non-selective agars (respectively *Brilliance* CCI and TSYEA),
- Seeding protocol with lyophilized strains or air-dried strains for dehydrated products.
- Seeding protocol for cleaning and process waters.

Table 40 gives the distribution of the positive samples per level of contamination and per thermal cycler used.

Table 40: distribution of the positive samples per contamination protocol and inoculation level (cl: contamination level in CFU/test portion)

Study	Positive samples	Naturally contaminated samples	Artificially contaminated samples			
			Spiking	Seeding		
			cl≤5 CFU	cl ≤ 3	3 < cl ≤ 10	cl > 10
Validated	128	6	23	79	20	0
Extension (2023)	61	0	12	49	0	0
Total	**189**	**3.2%**	**18.5%**	**67.7%**	**10.6%**	**0%**

In this extension, 61 samples gave a positive result. No naturally contaminated samples was detected. In total, all validations combined, 189 samples gave a positive result by at least one of the methods and 3.2% of them were naturally contaminated.

4.1.4. Results

Raw data are shown in Appendix K.

Tables 41 and 42 show the results of the sensitivity study for all categories, according to the thermal cycler used.

Table 41: interpretation of the sensitivity study results (R+/-: reference method positive or negative, A+/-: alternative method positive or negative, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation) – 7500 Fast

Study	Category	Response	R+	R-
Extension	PIF375	A+	PA = 16	PD = 11
		A-	ND = 3 incl. 0 PPND	NA = 35 incl. 2 PPNA
	ENV	A+	PA = 22	PD = 3
		A-	ND = 6 incl. 0 PPND	NA = 42 incl. 0 PPNA
	Total	A+	**PA = 38**	**PD = 14**
		A-	**ND = 9 incl. 0 PPND**	**NA = 77 incl.2 PPNA**

Table 42: interpretation of the sensitivity study results (R+/-: reference method positive or negative, A+/-: alternative method positive or negative, PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumptive positive before confirmation) – QS5

Study	Category	Response	R+	R-
Extension	PIF375	A+	PA = 16	PD = 11
		A-	ND = 3 incl. 0 PPND	NA = 35 incl. 2 PPNA
	ENV	A+	PA = 22	PD = 3
		A-	ND = 6 incl. 0 PPND	NA = 42 incl. 0 PPNA
	Total	A+	**PA = 38**	**PD = 14**
		A-	**ND = 9 incl. 0 PPND**	**NA = 77 incl.2 PPNA**

4.1.5. Calculation of relative trueness (RT), sensitivity (SE) and false positive ratio (PFR)

The set of results obtained were used to calculate the relative trueness, the sensitivity and the false positive ratio for each of the categories and for all the categories, according to the formulas set out in the EN ISO 16140-2:2016 standard.

This interpretation is shown below in Table 43 for the 7500 Fast thermal cycler and in Table 44 for the Quant Studio 5 thermal cycler.

The calculations formulae of the ISO 16140-2 standard are presented below:

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

Table 43: values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SEalt : sensitivity for the alternative method, SEref : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method) – 7500 Fast thermal cycler

Study	Cat.		Type	PA	NA	PD	ND	N	PPND*	PPNA*	SE_{alt} %	SE_{ref} %	RT%	FPR %
Extension	PIF375	a	Infant formula & Infant cereals with probiotics	8	10	3	0	21	0	1	100.0	72.7	86.4	10.0
		b	Infant formula & Infant cereals w/o probiotics	3	15	4	2	24	0	0	77.8	55.6	75.0	0.0
		c	Ingredients (caseinates, lecithin, etc...)	5	10	4	1	20	0	1	90.0	60.0	76.2	10.0
			Total	16	35	11	3	65	0	2	90.0	63.3	78.8	79.1
	ENV	a	Dusts and Residues	6	12	1	1	20	0	0	87.5	87.5	90.0	0.0
		b	Cleaning and Process Waters	6	15	2	3	26	0	0	72.7	81.8	80.8	0.0
		c	Surface samples	10	15	0	2	27	0	0	83.3	100.0	92.6	0.0
			Total	22	42	3	6	73	0	0	80.6	90.3	87.7	0.0
	Total			**38**	**77**	**14**	**9**	**138**	**0**	**2**	**85.2**	**77.0**	**83.6**	**2.5**

**PPNA are including in NA. PPND are including in ND*

Table 44: values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SEalt : sensitivity for the alternative method, SEref : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method) – QS5 thermal cycler

Study	Cat.		Type	PA	NA	PD	ND	N	PPND*	PPNA*	SE_{alt} %	SE_{ref} %	RT%	FPR %
Extension	PIF375	a	Infant formula & Infant cereals with probiotics	8	10	3	0	21	0	1	100.0	72.7	86.4	10.0
		b	Infant formula & Infant cereals w/o probiotics	3	15	4	2	24	0	0	77.8	55.6	75.0	0.0
		c	Ingredients (caseinates, lecithin, etc...)	5	10	4	1	20	0	1	90.0	60.0	76.2	10.0
			Total	16	35	11	3	65	0	2	90.0	63.3	79.1	5.4
	ENV	a	Dusts and Residues	6	12	1	1	20	0	0	87.5	87.5	90.0	0.0
		b	Cleaning and Process Waters	6	15	2	3	26	0	0	72.7	81.8	80.8	0.0
		c	Surface samples	10	15	0	2	27	0	0	83.3	100.0	92.6	0.0
			Total	22	42	3	6	73	0	0	80.6	90.3	87.7	0.0
	Total			**38**	**77**	**14**	**9**	**138**	**0**	**2**	**85.2**	**77.0**	**83.6**	**2.5**

**PPNA are including in NA. PPND are including in ND*

The results for the category of the extension study for the 2 categories tested, per kind of thermal cycler are summarized in Table 45.

Table 45: summary of the extension results per instrument

Parameter	7500 Fast	Quant Studio 5
	All categories	All categories
Sensitivity of the alternative method (SE_{alt})	85.2%	85.2%
Sensitivity of the reference method (SE_{ref})	77.0%	77.0%
Relative trueness (RT)	83.5%	83.6%
False positive ratio (FPR)	1.3%	2.5%

4.1.6. Analysis of the discordant results

Discordant results are examined according to the standard ISO 16140-2:2016.

The discordant results for this extension are presented in the following tables:

- Table 46 : negative deviations for 7500 FAST instrument,
- Table 47: positive deviations for 7500 FAST instrument,
- Table 48 : negative deviations for QS5 instrument,
- Table 49: positive deviations for QS5 instrument.

Table 46: summary of the negative deviations for the categories PIF375 and ENV for 7500 FAST instrument

								Reference method				BPW									
								ISO 22964[a]				18h at 34-38°C									
Cat.	Type	#	Sample	Contamination				CCI After CSB	Confirmatory tests		Final result	PCR result (Cq)	Confirmation				Final result	Agreement	ISO conf.		
				Strain		Conta.	Level CFU		OX	Gallery		7500 Fast	CCI 41,5°C	Oxydase	Gallery without purification	Gallery with purification	7500 Fast	7500 Fast	CCI after CSB	Ox.	Gallery
PIF375	b+	2428660	PIF 1st age batch 2	*C. sakazakii*	HXN562	Lyophilisation	2.2	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2498708	Infant formula for 6 at 12 months old	*C. sakazakii*	HXN562	Lyophilisation	2.0	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	c+	2392616	Whey	*C. sakazakii*	JBU888	Air-dried	1.8	+ (AM)	-	*Cronobacter sp*	P	-	-(EM)	/	/	/	A	ND	- (ø)	/	/
ENV.	a+	2483264	Dust suction filter weighing powders	*C. sakazakii*	HXA137	Air-dried	2.2	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2483199	Dosing water ligne 2 R1	*C. sakazakii*	HXK792	Seeding	1.8	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2483200	Rinsing water ligne 1 barrell emptying	*C. sakazakii*	HZP119	Seeding	1.8	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2483203	Rinsing water milk tank 2	*C. sakazakii*	JFU886	Seeding	1.6	+ (AM)	-	*Cronobacter sp*	P	-	- (EM)	/	/	/	A	ND	- (EM)	/	/
	c+	2483189	Wipe interior small tank before washing	*C. turincensis*	BTYS37	Spiking	2.2	+ (BM)	-	*Cronobacter sp*	P	-	- (EM)	/	/	/	A	ND	- (EM)	/	/
	c+	2483190	Wipe dairy floor	*C. sakazakii*	HXK792	Spiking	4.8	+ (DM)	-	*Cronobacter sp*	P	-	- (EM)	/	/	/	A (FN)	ND	+ (BM)	-	*Cronobacter sp*

Table 47: summary of the positive deviations for the categories PIF375 and ENV for 7500 FAST instrument

									Reference method				BPW									
									ISO 22964*				18h at 34-38°C									
					Contamination					Confirmatory tests			PCR result (Cq)	Confirmation				Final result	Agreement	ISO conf.		
Cat.	Type	#	Sample	Probiotics	Strain		Conta.	Level CFU	CCI After CSB	OX	Gallery	Final result	7500 Fast	CCI	Oxydase	Gallery without purification	Gallery with purification	7500 Fast	7500 Fast	CCI after CSB	Ox.	Gallery
PIF375	a+	2483214	Infant formula 1st age	*Bifidobacterium lactis* 5.1 10^7 CFU/g	*C. dublinensis*	GVV828	Lyophilisation	1.6	- (ø)	/	/	A	+ 22,54	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	a+	2483216	Infant formula 2nd age	*Bifidobacterium infantis* 5.8 10^6 CFU/g	*C. sakazakii*	HYD216	Lyophilisation	2.0	- (ø)	/	/	A	+ 20,60	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	a+	2428655	Cereals with quinoa, banana, prun	*B.lactis 3,4 10^5 CFU/g*	*C. sakazakii*	RLP329	Air-dried	2.0	- (ø)	/	/	A	+ 28.32	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2428663	Infant cereals brioche - cocoa	/	*C. muytjensi*	GVW884	Air-dried	2.5	- (EM)	/	/	A	+ 27.94	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (BM)	-	*Cronobacter sp*
	b+	2498707	Infant cereals cocoa & wheat	/	*C. sakazakii*	JGG241	Lyophilisation	2.6	- (ø)	/	/	A	+ 34,20	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2498730	Infant formula for 1 at 3 years old batch 1	/	*C. dublinensis*	GVV828	Lyophilisation	2.4	- (ø)	/	/	A	+ 38,12	+ (AL)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2498731	Infant formula for 1 at 3 years old batch 2	/	*C. sakazakii*	HXN562	Lyophilisation	2.4	- (ø)	/	/	A	+ 38,30	+ (AL)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	c+	2483276	Skimmed milk powder	/	*C. sakazakii*	HYD216	Lyophilisation	2.0	- (ø)	/	/	A	+ 37.07	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	c+	2392614	Wheat flour	/	*C. malonaticus*	HSE385	Air-dried	2.4	- (EM)	/	/	A	+ 32.89	+ (DM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (CM)	-	*Cronobacter sp*
	c+	2392615	Maltodextrin	/	*C. sakazakii*	JBU888	Air-dried	1.8	- (ø)	/	/	A	+ 25.39	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	c+	2498709	Powdered whole milk	/	*C. sakazakii*	JGG241	Lyophilisation	1.3	- (ø)	/	/	A	+ 38,98	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
ENV.	a+	2483263	Dusts vacuum PIF industry	/	*C. dublinensis*	DSEL33	Air-dried	3.0	- (ø)	/	/	A	+ 28.45	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2483198	Process water Ligne 2 R1	/	*C. turincensis*	HZN090	Seeding	2.0	- (ø)	/	/	A	+ 24.48	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2498734	Rinsing water TL+line TL-TR SAEQI	/	*C. sakazakii*	JFU886	Seeding	3.0	- (EM)	/	/	A	+ 26.07	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*

Table 48: summary of the negative deviations for the categories PIF375 and ENV for QS5 instrument

								Reference method				BPW									
								ISO 22964[■]				18h at 34-38°C									
Cat.	Type	#	Sample	Contamination				CCI After CSB	Confirmatory tests		Final result	PCR result (Cq)	Confirmation				Final result	Agreement	ISO conf.		
				Strain		Conta.	Level CFU		OX	Gallery		QS5	CCI 41,5°C	Oxydase	Gallery without purification	Gallery with purification	QS5	QS5	CCI after CSB	Ox.	Gallery
PIF375	b+	2428660	PIF 1st age batch 2	*C. sakazakii*	HXN562	Lyophilisation	2.2	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/		A	ND	- (ø)	/	/
	b+	2498708	Infant formula for 6 at 12 months old	*C. sakazakii*	HXN562	Lyophilisation	2.0	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	c+	2392616	Whey	*C. sakazakii*	JBU888	Air-dried	1.8	+ (AM)	-	*Cronobacter sp*	P	-	-(EM)	/	/	/	A	ND	- (ø)	/	/
ENV.	a+	2483264	Dust suction filter weighing powders	*C. sakazakii*	HXA137	Air-dried	2.2	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2483199	Dosing water ligne 2 R1	*C. sakazakii*	HXK792	Seeding	1.8	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2483200	Rinsing water ligne 1 barrell emptying	*C. sakazakii*	HZP119	Seeding	1.8	+ (AM)	-	*Cronobacter sp*	P	-	- (ø)	/	/	/	A	ND	- (ø)	/	/
	b+	2483203	Rinsing water milk tank 2	*C. sakazakii*	JFU886	Seeding	1.6	+ (AM)	-	*Cronobacter sp*	P	-	- (EM)	/	/	/	A	ND	- (EM)	/	/
	c+	2483189	Wipe interior small tank before washing	*C. turincensis*	BTYS37	Spiking	2.2	+ (BM)	-	*Cronobacter sp*	P	-	- (EM)	/	/	/	A	ND	- (EM)	/	/
	c+	2483190	Wipe dairy floor	*C. sakazakii*	HXK792	Spiking	4.8	+ (DM)	-	*Cronobacter sp*	P	-	- (EM)	/	/	/	A (FN)	ND	+ (BM)	-	*Cronobacter sp*

Table 49: summary of the positive deviations for the categories PIF375 and ENV for QS5 instrument

									Reference method ISO 22964*				BPW 18h at 34-38°C									
Cat.	Type	#	Sample	Probiotics	Contamination				CCI After CSB	Confirmatory tests		Final result	PCR result (Cq)	Confirmation				Final result	Agreement	ISO conf.		
					Strain		Conta.	Level CFU		OX	Gallery		QS5	CCI	Oxydase	Gallery without purification	Gallery with purification	QS5	QS5	CCI after CSB	Ox.	Gallery
PIF375	a+	2483214	Infant formula 1st age	*Bifidobacterium lactis* 5.1 10^7 CFU/g	*C. dublinensis*	GVV828	Lyophilisation	1.6	- (ø)	/	/	A	+ 22,24	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	a+	2483216	Infant formula 2nd age	*Bifidobacterium infantis* 5.8 10^6 CFU/g	*C. sakazakii*	HYD216	Lyophilisation	2.0	- (ø)	/	/	A	+ 19,16	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	a+	2428655	Cereals with quinoa, banana, prun	*B.lactis 3,4 10^5 CFU/g*	*C. sakazakii*	RLP329	Air-dried	2.0	- (ø)	/	/	A	+ 21.03	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2428663	Infant cereals brioche - cocoa	/	*C. muytjensi*	GVW884	Air-dried	2.5	- (EM)	/	/	A	+ 25.21	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (BM)	-	*Cronobacter sp*
	b+	2498707	Infant cereals cocoa & wheat	/	*C. sakazakii*	JGG241	Lyophilisation	2.6	- (ø)	/	/	A	+ 29,56	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2498730	Infant formula for 1 at 3 years old batch 1	/	*C. dublinensis*	GVV828	Lyophilisation	2.4	- (ø)	/	/	A	+ 34,93	+ (AL)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2498731	Infant formula for 1 at 3 years old batch 2	/	*C. sakazakii*	HXN562	Lyophilisation	2.4	- (ø)	/	/	A	+ 35,39	+ (AL)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	c+	2483276	Skimmed milk powder	/	*C. sakazakii*	HYD216	Lyophilisation	2.0	- (ø)	/	/	A	+ 35.47	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	c+	2392614	Wheat flour	/	*C. malonaticus*	HSE385	Air-dried	2.4	- (EM)	/	/	A	+ 28.02	+ (DM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (CM)	-	*Cronobacter sp*
	c+	2392615	Maltodextrin	/	*C. sakazakii*	JBU888	Air-dried	1.8	- (ø)	/	/	A	+ 20.60	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	c+	2498709	Powdered whole milk	/	*C. sakazakii*	JGG241	Lyophilisation	1.3	- (ø)	/	/	A	+ 35,02	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
ENV.	a+	2483263	Dusts vacuum PIF industry	/	*C. dublinensis*	DSEL33	Air-dried	3.0	- (ø)	/	/	A	+ 25.89	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2483198	Process water Ligne 2 R1	/	*C. turincensis*	HZN090	Seeding	2.0	- (ø)	/	/	A	+ 19.74	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*
	b+	2498734	Rinsing water TL+line TL-TR SAEQI	/	*C. sakazakii*	JFU886	Seeding	3.0	- (EM)	/	/	A	+ 19.53	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	PD	+ (AM)	-	*Cronobacter sp*

- **7500 FAST instrument**:

Nine negative deviations were observed: all from artificially contaminated samples.
The presence of *Cronobacter* in the alternative method broth was not detected, even using the additional confirmation protocol of the ISO 16140-2 standard (subculture in CSB before streaking on *Brilliance* CCI) for eight of them.
For the sample n°2483190 "Wipe dairy floor", the presence of *Cronobacter* in the alternative method broth has been detected after streaking on *Brilliance* CCI of the additional CSB broth.

Fourteen positive deviations were observed: all from artificially contaminated samples. For all the samples, the positive result obtained by PCR test was confirmed with direct streaking on *Brilliance* CCI agar plates.

- **QS5 instrument**:

Nine negative deviations were observed: all from artificially contaminated samples.
The presence of *Cronobacter* in the alternative method broth was not detected, even using the additional confirmation protocol of the ISO 16140-2 standard (subculture in CSB before streaking on *Brilliance* CCI) for eight of them.
For the sample n°2483190 "Wipe dairy floor", the presence of *Cronobacter* in the alternative method broth has been detected after streaking on *Brilliance* CCI of the additional CSB broth.

Fourteen positive deviations were observed: all from artificially contaminated samples. For all the samples, the positive result obtained by PCR test was confirmed with direct streaking on *Brilliance* CCI agar plates.

In conclusion, the negative deviations and the positive deviations most probably come from the nature of the study design. In an unpaired study, because of the difference of sampling between the two methods, and the use of seeded samples with low levels of contamination, no cell of *Cronobacter* may have been present in the sampling of one of the two methods.

4.1.7. Confirmations

One difference for sample n°2483272 “Soy lecithin” was observed between the two protocols used for the confirmations: direct streaking on *Brilliance* CCI gave a negative result but the streaking on *Brilliance* CCI after a subculture in CSB gave a positive result (confirm the positive PCR tests).
Two presumed positive not confirmed results were obtained during the extension study: one with the QS5 thermal cycler (2428654: cereals with biscuit with probiotics) and one with the 7500 FAST and QS5 thermal cycler (Soy lecithin). For these 2 samples, PCR retests were negative.

Three negative results (2483217: “Organic infant formula 2nd age with probiotics”, 2392617: “Lactose powder” and 2483190 : “Wipe dairy floor”) with the SureTect™ Cronobacter method haven’t been confirmed after direct streaking on *Brilliance* CCI or after subculture in CSB.

4.1.8. Calculation and interpretation of data

The following Tables shows the difference between negative deviations and positive deviations and the acceptability limits according to the kind of thermal cycler used:

- Table 50: 7500 Fast,
- Table 51: Quant Studio 5.

Table 50: acceptability limits for the 7500 Fast instrument

Category	Type	N+	ND	PD	Unpaired protocol	
					ND-PD	AL
PIF375	a	11	0	3	/	/
	b	9	2	4		
	c	10	1	4		
	Total	**30**	**3**	**11**	**-8**	**3**
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	2	0		
	Total	**31**	**6**	**3**	**3**	**3**
All categories		**61**	**9**	**14**	**-5**	**4**

Table 51: acceptability limits for the QS5 thermal cycler

Category	Type	N+	ND	PD	Unpaired protocol	
					ND-PD	AL
PIF375	a	11	0	3	/	/
	b	9	2	4		
	c	10	1	4		
	Total	**30**	**3**	**11**	**-8**	**3**
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	2	0		
	Total	**31**	**6**	**3**	**3**	**3**
All categories		**61**	**9**	**14**	**-5**	**4**

The observed values (ND – PD) are below the acceptability limits for each category and for all categories for the two kinds of thermal cyclers tested. The alternative method produces results comparable to the reference method.

4.1.9. Enrichment broth storage at 2 – 8°C for 72 hours

A stability study of the enriched broths stored at 5±3°C for 72 hours was performed on positive and discordant samples. After storage, the broths were reanalyzed and confirmed.

During this extension study, the conclusion for only one sample changed with QS5 instrument after storage at 2-8°C for 3 days: sample n°2483190 “Wipe dairy floor” which was negative just after enrichment and became positive after the cold storage.
The concordance between the reference and the alternative method moved from ND to PA.

The interpretation of the results after cold storage is presented in the following Tables according to the kind of thermal cycler used:

- Table 52: 7500 Fast,
- Table 53: Quant Studio 5.

Table 52: acceptability limits for the 7500 FAST thermal cycler after cold storage of the broths

Category	Type	N+	ND	PD	Unpaired protocol	
					ND-PD	AL
PIF375	a	11	0	3	/	/
	b	9	2	4		
	c	10	1	4		
	Total	30	3	11	-8	3
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	2	0		
	Total	31	6	3	3	3
All categories		61	9	14	-5	4

Table 53: acceptability limits for the QS5 thermal cycler after cold storage of the broths

Category	Type	N+	ND	PD	Unpaired protocol	
					ND-PD	AL
PIF375	a	11	0	3	/	/
	b	9	2	4		
	c	10	1	4		
	Total	30	3	11	-8	3
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	1	0		
	Total	31	5	3	2	3
All categories		61	8	14	-6	4

After cold storage of the enriched broths, the observed values (ND – PD) are below the acceptability limits for each category and for all categories for the two kinds of thermal cyclers tested.

The alternative method produces results comparable to the reference method.

4.1.10. PCR inhibitions

Three inhibitions were observed only with 7500 FAST instrument during this extension study.

All samples on which inhibitions were observed are listed in table 54, as well as the percentage of inhibition with regard to the number of samples analyzed.

Table 54: PCR inhibitions

Sample n°	Product	PCR result	Final result
		7500 Fast	
2428654	Cereals with biscuit with probiotics (3.6 10^5 CFU/g)	1st test : inh. Pur retest : + 39.56 1/10 retest: -	A (FP)
2428656	Cereals with rice with probiotics (10^7 CFU/g)	1st test : inh. Pur retest : - 1/10 retest: -	A
2498732	Infant cereals biscuit	1st test : inh. Pur retest : inh. 1/10 retest: + (28.37)	P
% of inhibition		**2.1 %**	

A new test on the pure enrichment broth and its 1/10th dilution was carried out for the 3 samples. For sample n°2428654, the enrichment broth retest gave a positive result, but no confirmation could show the presence of *Cronobacter*. For the samples n°2428656 and n°2498732, the protocol for managing inhibitions made it possible to obtain a result.
The percentage of inhibition for 7500 FAST instrument is 2.1% and 0.0% for QS5 instrument.

4.2. Relative level of detection study

4.2.1. Matrix-strain pairs

Table 55 summarizes the matrix-strain pairs tested and the kind of thermal cyclers on which it was analyzed.

Table 55: matrix-strain pairs for the determination of the RLOD

Category	Matrix	Inoculated strain	Storage conditions before analysis	Thermal cycler tested
Infant formula, infant cereals and related ingredients (up to 375 g sample size) **PIF375**	Infant formula with probiotics	*Cronobacter sakazakii JBU888,* wild strain isolated from a milk powder	Air-dried strain with stabilization after inoculation for 2 weeks at room temperature	7500 Fast QS5
Production environmental samples **ENV**	Surface sample : Stainless steel 1''x 1''	*Cronobacter turicensis HZN090,* wild strain isolated from dairy environment	Seeding protocol	7500 Fast QS5

4.2.2. Contamination protocol

The Infant formula with probiotics was contaminated using a seeding protocol: bulk contaminations was performed on the matrix for the different levels of contamination, then the matrix was stored at ambient temperature for two weeks before analysis.
Three levels of contamination were prepared consisting of a negative control level, a low level, and a higher level.
The negative control level shall not produce positive results. Five replicates were tested for this level.
The low level shall be the theoretical detection level, it was contaminated at 0.7 - 1 CFU per test portion to obtain fractional recovery results. Twenty replicates were tested for this level.
The higher level shall be just above the theoretical detection level, it was contaminated at 2 - 3 CFU per test portion. Five replicates were tested for this level.

For the 1"x1" stainless steel, the low level was contaminated at a range of 10^4 CFU/surface area and the high level at a range of 10^6 CFU/surface area. After drying at room temperature for 16 to 24 hours, the surfaces were swabbed, and the swabs placed in a neutralizer 2 hours before being analyzed.
The negative control level shall not produce positive results. Five replicates were tested for this level.
Twenty replicates were tested for the low level and five replicates for the high level.

For the two items, samples were analyzed by the reference method (test portions of 10 g or surface) and the alternative method (test portions of 375 g or surface).

4.2.3. Results

The detailed results table is set out in Appendix L for the 2 categories tested during the extension study.

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method: RLOD= LOD_{alt} / LOD_{ref}.
The RLODs calculations were performed according to the standard ISO 16140-2: 2016 using the Excel spreadsheet available for download at http://standards.iso.org/iso/16140, with unknown concentrations. Values of the RLODs are set out in table 56 for 7500 FAST instrument and in table 57 for QS5 instrument.

Table 56: RLODs values for all categories with the 7500 Fast thermal cycler (RLOD: the estimated relative level of detection value, RLODU: the upper limit of the 95% confidence interval for RLOD, RLODL: the lower limit of the 95% confidence interval for RLOD)

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value	AL
PIF 375 g with probiotics	1.888	0.537	6.635	0.636	0.628	1.012	0.312	**2.5**
Environmental sample	1.000	0.339	2.948	0.000	0.541	0.000	1.000	**2.5**
Combined	**1.257**	**0.604**	**2.614**	**0.228**	**0.366**	**0.624**	**0.533**	**/**

Table 57: RLODs values for all categories with the QS5 thermal cycler (RLOD: the estimated relative level of detection value, RLODU: the upper limit of the 95% confidence interval for RLOD, RLODL: the lower limit of the 95% confidence interval for RLOD)

Name	RLOD	RLODL	RLODU	b=ln(RLOD)	sd(b)	z-Test statistic	p-value	AL
PIF 375 g with probiotics	1.888	0.537	6.635	0.636	0.628	1.012	0.312	**2.5**
Environmental sample	1.000	0.339	2.948	0.000	0.541	0.000	1.000	**2.5**
Combined	**1.257**	**0.604**	**2.614**	**0.228**	**0.366**	**0.624**	**0.533**	**/**

4.2.4. Interpretation and conclusion

The RLODs values are below the acceptability limit set at 2.5 for “unpaired” categories described in ISO 16140-2:2016.

4.2.5. Expression of the LOD values

The LOD_{50} calculations according to Wilrich & Wilrich POD-LOD calculation program - version 11, are given in table 58.
For the environmental sample category, the spreadsheet used does not allow an $LOD_{50\%}$ to be calculated with the contamination protocol used.

Table 58: $LOD_{50\%}$ for the alternative and reference method in CFU/test portion

Categories	Reference method ISO 22964	Alternative method	
		7500 Fast	Quant Studio 5
PIF375 with probiotics	2.860	5.292	5.292

For the PIF 375g category, the tests carried out during the RLOD study produced few positive results both in the ISO method and in the alternative method, but in sufficient number to determine an

LOD_{50}. Therefore, the value of this calculated LOD_{50} is high for the alternative method (5.292). In the context of method verification according to the ISO 16140-3 standard, it is recommended to use protocol 3 proposed by the standard in order to determine the $eLOD_{50}$

4.2.6. Conclusion of the extension

The extension study of the methods was performed according to the EN ISO 16140-2:2016 standard. Tests were performed with 2 thermal cyclers 7500 Fast and Quant Studio 5 PCR instruments.

- **Sensitivity study**

During this extension, the performance of the SureTect™ Cronobacter method was compared to that of the ISO 22964 reference method by analyzing 138 samples divided into 2 categories.
The observed values (ND – PD) were below the acceptability limits for each category and for all categories after the initial test and after three days of conservation at 5±3°C.
Statistically, the new protocols proposed for the 2 categories tested for alternative method produces results comparable to that of the reference method.

- **Relative level of detection study**

The relative detection level of the SureTect™ Cronobacter method and reference method was evaluated by artificially contaminating matrices of the application scope.
The SureTect™ Cronobacter method and the reference method showed similar LODs values for the detection of *Cronobacter* spp in the categories tested.

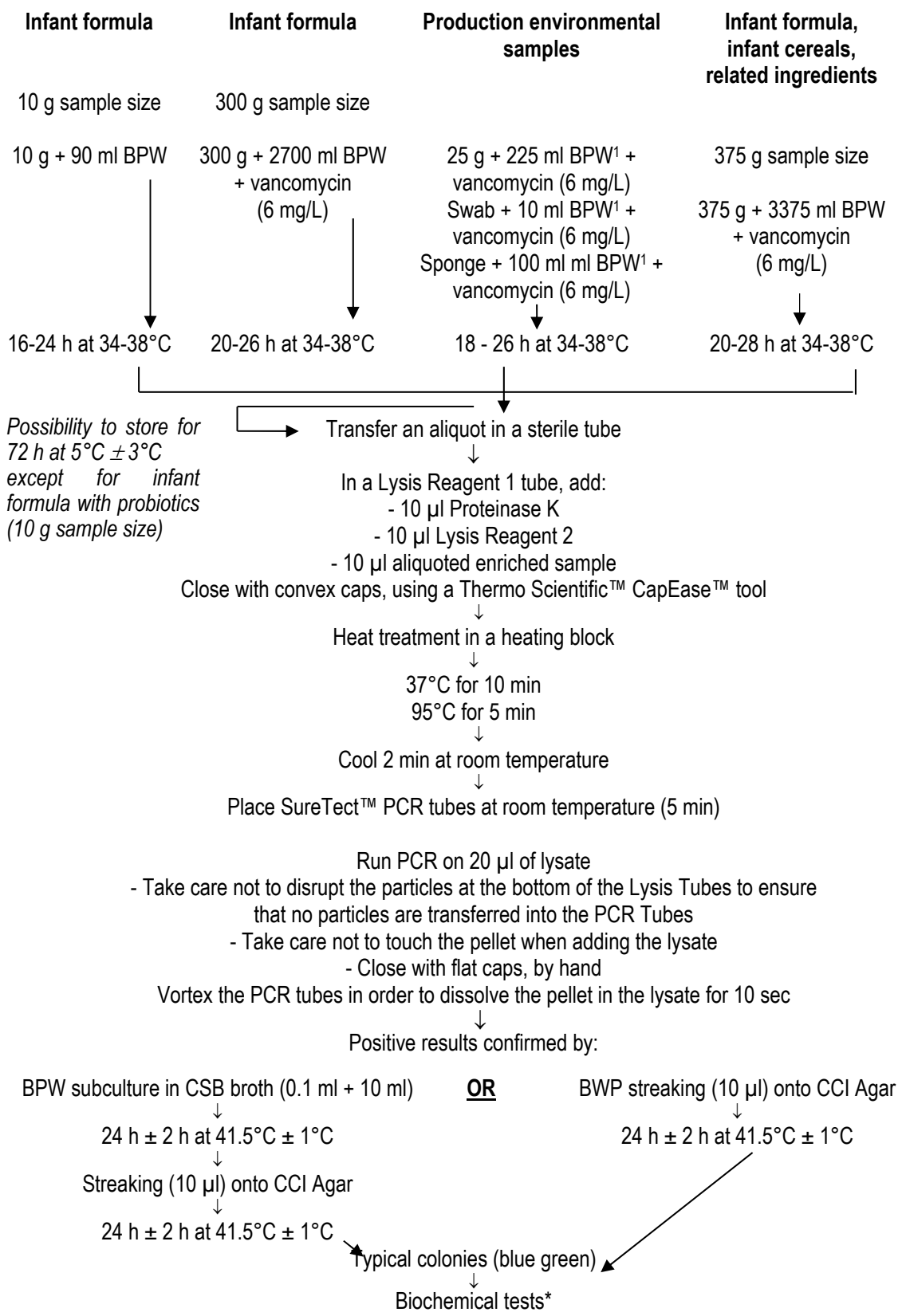
5. General conclusion

The data and the interpretation of the method comparison study, interlaboratory study and extension study fulfill the requirements of the standard EN ISO 16140-2:2016. The Thermo Scientific™ SureTect™ *Cronobacter* species PCR Assay method for the detection of *Cronobacter* spp. is considered as equivalent to the standard EN ISO 22964:2017.

Le Lion d'Angers, October 17th , 2023
François Le Nestour
Head of the Microbiology Department

APPENDICES

Appendix A – Flow diagram of the alternative method: Thermo Scientific™ SureTect™ *Cronobacter* species PCR Assay for the detection of *Cronobacter spp*



*According to ISO rules, it as well possible to use ISO 16140-6:2019 validated confirmation method.

[1] For sampling after cleaning process premoisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml BPW)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml BPW)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml BPW)

Appendix B – Flow diagram of the reference method: ISO 22964 (April 2017) - Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

Infant formula and ingredients:

10 g + 90 mL BPW

Environmental samples[2]:

1 swab + 10 mL BPW [2]

or 1 sponge + 100 mL BPW [2]

or 10 g + 90 mL BPW[2]

or 10 mL

↓

Incubation 18 h ± 2 h at 34 – 38°C

↓

0.1 mL + 10 mL CSB

↓

Incubation 24 h ± 2 h at 41.5°C ± 1°C

↓

Streaking onto CCI Agar plate

↓

Incubation 24 h ± 2 h at 41.5°C ± 1°C

↓

Confirmatory test on one typical colony, and four other colonies

(if the first one is negative (typical colony: blue to blue-green))

Streaking onto TSA

↓

Incubation 18 h – 24 h at 37°C ± 1°C

↓

Biochemical confirmation (oxidase, mini-galleries)

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[2] For sampling after cleaning process premoisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml BPW)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml BPW)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml BPW)

Appendix C - Artificial contamination of the samples – Initial validation study

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
2310	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (3,6.10⁶/g)**	*Cronobacter sakazakii Ad 1446*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3,8	-	1	a
4885	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 704*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,3	-	1	a
4886	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 704*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,3	-	1	a
4887	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 831*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1,1	-	1	a
4888	**Poudre de lait infantile 0-36 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 831*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1,1	-	1	a
4889	**Poudre de lait infantile croissance**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 916*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,3	+	1	a
4890	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 916*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,3	-	1	a
4891	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1418*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1	+	1	a
4892	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1446*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,4	+	1	a
4893	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1446*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,4	+	1	a
4894	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1446*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,4	+	1	a
4895	**Poudre de lait infantile croissance**	**Infant formula milk powder**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-0-1 (0,2)	-	1	a
4896	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-0-1 (0,2)	-	1	a

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
4897	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-0-1 (0,2)	-	1	a
4898	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter dublinensis subsp lactaridi E791*	/	Spiking-HT 56°C 8 min	>0,90	0-1-0-3-0 (0,8)	-	1	a
4899	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter dublinensis subsp lactaridi E791*	/	Spiking-HT 56°C 8 min	>0,90	0-1-0-3-0 (0,8)	-	1	a
4900	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^4/g)**	*Cronobacter dublinensis subsp lactaridi E791*	/	Spiking-HT 56°C 8 min	>0,90	0-1-0-3-0 (0,8)	-	1	a
4901	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,8.10^4/g)**	*Cronobacter dublinensis subsp lactaridi E791*	/	Spiking-HT 56°C 8 min	>0,90	0-1-0-3-0 (0,8)	-	1	a
4902	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<200/g)**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-0-1 (0,2)	-	1	a
4903	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^6/g)**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-0-1 (0,2)	-	1	a
5070	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1437*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	2,5	+	1	a
5071	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1437*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	2,5	+	1	a
5072	**Poudre de lait infantile 1-3 ans**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1437*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	2,5	+	1	a
5073	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1437*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	2,5	+	1	a
5074	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 1437*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	2,5	+	1	a
5075	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 940*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	-	1	a
5076	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 940*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	+	1	a
5077	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 940*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	-	1	a

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
5078	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 893*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	0,6	+	1	a
5079	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 893*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	0,6	+	1	a
5080	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 893*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	0,6	+	1	a
5081	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	*Cronobacter sakazakii Ad 893*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	0,6	+	1	a
5082	**Poudre de lait infantile avec probiotiques-croissance**	**Infant formula milk powder with probiotics (<200/g)**	*Cronobacter sakazakii Ad 940*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	-	1	a
5206	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,4.10^{3} cfu/g)**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	0,55	0-0-0-2-3 (1,0)	-	1	a
5207	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,0.10^{2} cfu /g)**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	0,55	0-0-0-2-3 (1,0)	-	1	a
5208	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^{2} cfu /g)**	*Cronobacter malonaticus E752*	Baby food	Spiking-HT 56°C 8 min	0,55	0-0-0-2-3 (1,0)	-	1	a
5240	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (7,9.10^{6} cfu /g)**	*Cronobacter sakazakii Ad 1707*	Environment dairy industry	Seeding-Lyophilized strain, 1 week at 20°C	/	3,0	+	1	a
5241	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^{4} cfu /g)**	*Cronobacter sakazakii Ad 1437*	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,5	+	1	a
5242	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,0.10^{4} cfu /g)**	*Cronobacter sakazakii Ad 940*	Dairy product	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	+	1	a
1455	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,0.10^{4}/g)**	*Cronobacter sakazakii Ad 704*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	+	1	b

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
1456	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (8,0.10^4/g)**	*Cronobacter sakazakii Ad 704*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	+	1	b
1457	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,6.10^6/g)**	*Cronobacter sakazakii Ad 704*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	-	1	b
1458	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,0.10^5/g)**	*Cronobacter sakazakii Ad 831*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,4	+	1	b
1459	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (5,8.10^7/g)**	*Cronobacter sakazakii Ad 831*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,4	-	1	b
1460	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	*Cronobacter sakazakii Ad 831*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,4	+	1	b
1461	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	*Cronobacter sakazakii Ad 916*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,2	+	1	b
1462	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	*Cronobacter sakazakii Ad 916*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,2	-	1	b
1463	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	*Cronobacter sakazakii Ad 916*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,2	-	1	b
1464	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	*Cronobacter sakazakii Ad 1418*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,3	-	1	b
1465	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,2.10^6/g)**	*Cronobacter sakazakii Ad 1418*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,3	+	1	b
1466	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,3.10^7/g)**	*Cronobacter sakazakii Ad 1418*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,3	+	1	b

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
1467	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($1,5.10^6$/g)**	*Cronobacter sakazakii Ad 1446*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,7	-	1	b
1468	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($4,0.10^4$/g)**	*Cronobacter sakazakii Ad 1446*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,7	-	1	b
1469	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($1,2.10^7$/g)**	*Cronobacter sakazakii Ad 1446*	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,7	-	1	b
2303	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($4,7.10^6$/g)**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,9	-	1	b
2304	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($5,4.10^6$/g)**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,9	+	1	b
2305	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($6,0.10^6$/g)**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,9	-	1	b
2306	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($7,0.10^5$/g)**	*Cronobacter sakazakii Ad 1418*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	4,1	+	1	b
2307	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($8,0.10^5$/g)**	*Cronobacter sakazakii Ad 1418*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	4,1	+	1	b
2308	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($3,2.10^5$/g)**	*Cronobacter sakazakii Ad 1418*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	4,1	+	1	b
2309	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics ($8,8.10^5$/g)**	*Cronobacter sakazakii Ad 1446*	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3,8	+	1	b
2049	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 893*	Dairy product	Seeding-48 h 4°C	/	1-3-3-7-5 (3,8)	+	2	a
2050	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 947*	Dairy product	Seeding-48 h 4°C	/	6-2-3-3-0 (2,8)	+	2	a

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
2051	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 1428*	Dairy product	Seeding-48 h 4°C	/	4-3-1-1-1 (2,0)	+	2	a
2052	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 1435*	Dairy product	Seeding-48 h 4°C	/	3-0-1-5-2 (2,2)	+	2	a
2053	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 947*	Dairy product	Seeding-48 h 4°C	/	6-2-3-3-0 (2,8)	+	2	a
2446	**Eau de process**	**Process water**	*Cronobacter sakazakii Ad 1708*	Environmen dairy industry	Spiking-HT 8 min 56°C	1,93	0-0-0-0-0 (0,0)	-	2	a
2447	**Eau de nettoyage**	**Cleaning water**	*Cronobacter sakazakii Ad 1708*	Environment dairy industry	Spiking-HT 8 min 56°C	1,93	0-0-0-0-0 (0,0)	-	2	a
4085	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 1437*	Dairy product	Spiking-pH4 and HT 8 min 56°C	0,60	0-0-1-0-2 (0,6)	-	2	a
2448	**Eau de syphon**	**Siphon water**	*Cronobacter sakazakii Ad 1428*	Dairy product	Spiking-pH4 during 7days	0,57	3-0-1-1-0 (1,0)	+	2	b
2677	**Poussièrrs laiterie**	**Dusts**	*Cronobacter sakazakii Ad 704*	Dairy product	Spiking-Lyophilizated strain	0,42	2-1-3-0-4 (2,0)	+	2	b
2678	**Poussières aspirateur**	**Dusts**	*Cronobacter sakazakii Ad 704*	Dairy product	Spiking-Lyophilizated strain	0,42	2-1-3-0-4 (2,0)	+	2	b
2679	**Déchets poudre de lait**	**Dusts**	*Cronobacter sakazakii Ad 916*	Dairy product	Spiking-Lyophilizated strain	0,48	2-3-1-4-4 (2,8)	+	2	b
2680	**Poussières laiterie**	**Dusts**	*Cronobacter sakazakii Ad 916*	Dairy product	Spiking-Lyophilizated strain	0,48	2-3-1-4-4 (2,8)	+	2	b
2681	**Poussières aspirateur**	**Dusts**	*Cronobacter sakazakii Ad 831*	Dairy product	Spiking-Lyophilizated strain	0,48	0-0-2-3-2 (1,4)	+	2	b
2682	**Déchets poudre de lait**	**Dusts**	*Cronobacter sakazakii Ad 831*	Dairy product	Spiking-Lyophilizated strain	0,48	0-0-2-3-2 (1,4)	+	2	b
3479	**Eponge poussières (industrie PDL)**	**Dusts sponge**	*Cronobacter sakazakii Ad 1437*	Milk powder	Spiking-pH4 during 7days	0,52	1-0-0-2-0 (0,6)	-	2	b
3480	**Eponge poussières (industrie PDL)**	**Dusts sponge**	*Cronobacter sakazakii Ad 893*	Milk powder	Spiking-pH4 during 7days	0,41	0-1-0-0-0 (0,2)	-	2	b
3481	**Eponge poussières (industrie PDL)**	**Dusts sponge**	*Cronobacter sakazakii Ad 940*	Milk powder	Spiking-pH4 during 7days	0,56	4-3-3-4-8 (4,4)	+	2	b

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
2055	**Lingette étagère salle de stockage poudre de lait**	**Wipe**	*Cronobacter sakazakii Ad 947*	Dairy product	Seeding-48 h 4°C	/	6-2-3-3-0 (2,8)	+	2	c
2056	**Lingette étagère salle de stockage poudre de lait**	**Wipe**	*Cronobacter sakazakii Ad 1428*	Dairy product	Seeding-48 h 4°C	/	4-3-1-1-1 (2,0)	+	2	c
2057	**Lingette plan de travail salle de stockage poudre de lait**	**Wipe**	*Cronobacter sakazakii Ad 1435*	Dairy product	Seeding-48 h 4°C	/	3-0-1-5-2 (2,2)	+	2	c
2058	**Lingette plan de travail salle de stockage poudre de lait**	**Wipe**	*Cronobacter sakazakii Ad 2290*	Dairy product	Seeding-48 h 4°C	/	0-1-1-1-2 (1,0)	+	2	c
2440	**Lingette transport pneumatique avant nettoyage**	**Wipe**	*Cronobacter sakazakii Ad 1708*	Environment dairy industry	Spiking-HT 8 min 56°C	1,93	0-0-0-0-0 (0,0)	+	2	c
2441	**Lingette sol**	**Wipe**	*Cronobacter sakazakii Ad 1708*	Environment dairy industry	Spiking-HT 8 min 56°C	1,93	0-0-0-0-0 (0,0)	+	2	c
2443	**Lingette transport pneumatique après nettoyage**	**Wipe**	*Cronobacter sakazakii Ad 1428*	Dairy product	Spiking-pH4 during 7days	0,57	3-0-1-1-0 (1,0)	+	2	c
2444	**Lingette sol après nettoyage**	**Wipe**	*Cronobacter sakazakii Ad 1428*	Dairy product	Spiking-pH4 during 7days	0,57	3-0-1-1-0 (1,0)	+	2	c
2445	**Lingette porte tour séchage après nettoyage**	**Wipe**	*Cronobacter sakazakii Ad 1428*	Dairy product	Spiking-pH4 during 7days	0,57	3-0-1-1-0 (1,0)	+	2	c
3339	**Ecouvillon douchette réserve**	**Swab**	*Cronobacter sakazakii Ad 953*	Dairy product	Spiking-Lyophilizated strain	0,40	0-0-2-2-1 (1,0)	+	2	c
3340	**Ecouvillon dilumate**	**Swab**	*Cronobacter sakazakii Ad 1446*	Infant formula	Spiking-Lyophilizated strain	0,56	2-1-1-3-1 (1,6)	+	2	c
3341	**Ecouvillon E5**	**Swab**	*Cronobacter sakazakii Ad 953*	Dairy product	Spiking-Lyophilizated strain	0,40	0-0-2-2-1 (1,0)	-	2	c
3342	**Ecouvillon 35**	**Swab**	*Cronobacter sakazakii Ad 1446*	Infant formula	Spiking-Lyophilizated strain	0,56	2-1-1-3-1 (1,6)	+	2	c
3477	**Eponge surface (industrie PDL)**	**Surface sponge**	*Cronobacter sakazakii Ad 893*	Milk powder	Spiking-pH4 during 7days	0,41	0-1-0-0-0 (0,2)	+	2	c

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
3478	**Eponge surface (industrie PDL)**	**Surface sponge**	*Cronobacter sakazakii Ad 940*	Milk powder	Spiking-pH4 during 7days	0,56	4-3-3-4-8 (4,4)	+	2	c
3265	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 831*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,6	+	3	a
3266	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 831*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,6	+	3	a
3267	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1446*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	<1,0	-	3	a
3268	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1446*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	a
3269	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 940*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	3,0	+	3	a
3270	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 940*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	3,0	+	3	a
3271	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 940*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	3,0	+	3	a
3272	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1420*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	a
3273	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1420*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	a
3483	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	1,1	5-2-1-0-0 (1,6)	+	3	a
3484	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	1,1	5-2-1-0-0 (1,6)	-	3	a
3485	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	1,1	5-2-1-0-0 (1,6)	-	3	a
3486	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	1-0-2-2-2 (1,4)	-	3	a
3487	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	1,1	5-2-1-0-0 (1,6)	-	3	a
3488	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	1-0-2-2-2 (1,4)	-	3	a

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
3489	**Poudre de lait infantile 1er âge avec probiotiques (<10cfu/g)**	**Infant formula with probiotics (<10cfu/g)**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	1-0-2-2-2 (1,4)	-	3	a
3490	**Poudre de lait infantile 2e âge avec probiotiques (1,1 10^6 cfu/g)**	**Infant formula with probiotics (1,1 10^6 cfu/g)**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	1-0-2-2-2 (1,4)	-	3	a
3753	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	0,5	0-0-0-3-1 (0,8)	-	3	a
3754	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	0,5	0-0-0-3-1 (0,8)	+	3	a
3755	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter dublinensis DSM18705*	Milk powder	Spiking-HT 8 min 56°C	0,5	0-0-0-3-1 (0,8)	-	3	a
3756	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	0-4-3-2-1 (2,0)	-	3	a
3757	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	0-4-3-2-1 (2,0)	-	3	a
3758	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter turicensis Ad 1445*	Infant formula	Spiking-HT 8 min 56°C	0,7	0-4-3-2-1 (2,0)	+	3	a
3759	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter malonaticus DSM18702*	Milk powder	Spiking-HT 8 min 56°C	0,5	1-6-3-4-3 (3,4)	+	3	a
4086	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 1437*	Dairy product	Spiking-pH4 and HT 8 min 56°C	0,60	0-0-1-0-2 (0,6)	-	3	a
4087	**Eau de rinçage**	**Rinsing water**	*Cronobacter sakazakii Ad 1437*	Dairy product	Spiking-pH4 and HT 8 min 56°C	0,60	0-0-1-0-2 (0,6)	-	3	a
4398	**Eau de rinçage production PDL**	**Rinsing water**	*Cronobacter turicensis Ad 1445*	Dairy product	Seeding-48 h 4°C	/	2-1-0-3-5 (2,2)	+	3	a
4399	**Eau de rinçage FOARMOR**	**Rinsing water**	*Cronobacter turicensis Ad 1445*	Dairy product	Seeding-48 h 4°C	/	2-1-0-3-5 (2,2)	+	3	a
1750	**Poudre de lait infantile 1er âge avec probiotiques (<10cfu/g)**	**Infant formula with probiotics (<10cfu/g)**	*Cronobacter sakazakii Ad 704*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	+	3	b
1751	**Poudre de lait infantile 1er âge avec probiotiques (<10cfu/g)**	**Infant formula with probiotics (<10cfu/g)**	*Cronobacter sakazakii Ad 704*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	+	3	b

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
1752	**Poudre de lait infantile 1er âge avec probiotiques (<10cfu/g)**	**Infant formula with probiotics (<10cfu/g)**	*Cronobacter sakazakii Ad 704*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	+	3	b
1753	**Poudre de lait infantile 1er âge avec probiotiques (1,5 10^5 cfu/g)**	**Infant formula with probiotics (1,5 10^5 cfu/g)**	*Cronobacter sakazakii Ad 916*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,8	+	3	b
1754	**Poudre de lait infantile 1er âge avec probiotiques (9,7 10^5 cfu/g)**	**Infant formula with probiotics(9,7 10^5 cfu/g)**	*Cronobacter sakazakii Ad 916*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,8	+	3	b
1755	**Poudre de lait infantile 3e âge avec probiotiques (8,4 10^4 cfu/g)**	**Infant formula with probiotics (8,4 10^4 cfu/g)**	*Cronobacter sakazakii Ad 916*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3,0	+	3	b
1756	**Poudre de lait infantile 1er âge avec probiotiques (1,0 10^6 cfu/g)**	**Infant formula with probiotics (1,0 10^6 cfu/g)**	*Cronobacter sakazakii Ad 916*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3,0	+	3	b
1757	**Poudre de lait infantile 1er âge avec probiotiques (2,2 10^5 cfu/g)**	**Infant formula with probiotics (2,2 10^5 cfu/g)**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	b
1758	**Poudre de lait infantile 2e âge avec probiotiques (1,1 10^6 cfu/g)**	**Infant formula with probiotics (1,1 10^6 cfu/g)**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	b
1759	**Poudre de lait infantile 1er âge avec probiotiques (1,0 10^6 cfu/g)**	**Infant formula with probiotics (1,0 10^6 cfu/g)**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	b
1760	**Poudre de lait infantile 2e âge avec probiotiques (20 cfu/g)**	**Infant formula with probiotics (20 cfu/g)**	*Cronobacter sakazakii Ad 1437*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1,2	-	3	b
1761	**Poudre de lait infantile 1er âge avec probiotiques (8,0 10^5 cfu/g)**	**Infant formula with probiotics (8,0 10^5 cfu/g)**	*Cronobacter sakazakii Ad 1437*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1,2	+	3	b
1762	**Poudre de lait infantile 1er âge avec probiotiques (<10cfu/g)**	**Infant formula with probiotics (<10cfu/g)**	*Cronobacter sakazakii Ad 1437*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1,2	+	3	b

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measure-ment	Inoculation level CFU/sample			
1763	**Poudre de lait infantile 1er âge avec probiotiques (6,0 10^5 cfu/g)**	**Infant formula with probiotics (6,0 10^5 cfu/g)**	*Cronobacter sakazakii Ad 1420*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	b
1764	**Poudre de lait infantile 1er âge avec probiotiques (3,0 10^5 cfu/g)**	**Infant formula with probiotics (3,0 10^5 cfu/g)**	*Cronobacter sakazakii Ad 1420*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1,0	+	3	b
3264	**Poudre de lait infantile 1er âge avec probiotiques (3,9 10^5 cfu/g)**	**Infant formula with probiotics (3,9 10^5 cfu/g)**	*Cronobacter sakazakii Ad 831*	Dairy product	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,6	+	3	b
3760	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter malonaticus DSM18702*	Milk powder	Spiking-HT 8 min 56°C	0,5	1-6-3-4-3 (3,4)	-	3	b
3761	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter malonaticus DSM18702*	Milk powder	Spiking-HT 8 min 56°C	0,5	1-6-3-4-3 (3,4)	+	3	b
3940	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3941	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 953*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3942	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1437*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3943	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1437*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3944	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 893*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3945	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 893*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3946	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 893*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b
3947	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1446*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	-	3	b
3948	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	*Cronobacter sakazakii Ad 1446*	Dairy product	Seeding, lyophilized strain, 2 weeks at 20°C	/	5,0	+	3	b

APPENDIX C - Artificial contamination - Extension study (2021)

Sample N°	Product (probiotics enumeration CFU/g)	Type	Result	Strain	Strain code	Seeding protocol		Contamination level (CFU/test portion)
2034825	Infant formula milk powder with probiotics (*Lactobacillus reuteri* 5,5.10^5 CFU/g)	a	Neg.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	2,6
2034826	Infant formula milk powder with probiotics (*Bifidobacterium infantis* 3,1.10^6 CFU/g)	a	Neg.	*Cronobacter malonaticus*	HSE835	Air-dried	2 weeks at ambient temperature	1,8
2034829	Infant formula milk powder with probiotics (*Lactobacillus fermentum hereditum* 1,0.10^6 CFU/g)	a	Neg.	*Cronobacter turicensis*	HZN090	Lyophilized	2 weeks at ambient temperature	2,6
2034842	Infant formula milk powder with probiotics (*Lactobacillus fermentum hereditum* 1,0.10^6 CFU/g)	a	Neg.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	1,6
2034843	Infant cereals with probiotics (biscuit) (*B. lactis* 7,0.10^5 CFU/g)	a	Neg.	*Cronobacter turicensis*	HZN090	Lyophilized	2 weeks at ambient temperature	2,6
2034844	Infant cereals with probiotics (honey) (*B. lactis* 3,4.10^6 CFU/g)	a	Neg.	*Cronobacter sakazakii*	JGG241	Lyophilized	2 weeks at ambient temperature	2,2
2034841	Infant cereals with probiotics (*B. lactis* 6,8.10^6 CFU/g)	a	Neg.	*Cronobacter sakazakii*	AWX553	Air-dried	2 weeks at ambient temperature	2,2
1978475	Infant formula milk powder with probiotics (*Lactobacillus reuteri* 4,0.10^5 CFU/g)	a	Neg.	*Cronobacter malonaticus*	HSE835	Air-dried	2 weeks at ambient temperature	1,8
2034846	Infant cereals with probiotics (5 cereals) (*B. lactis* 5,0.10^5 CFU/g)	a	Neg.	*Cronobacter turicensis*	HZN090	Air-dried	2 weeks at ambient temperature	2,0
2034847	Infant cereals with probiotics (chocolate & biscuit) (*B. lactis* 7,0.10^5 CFU/g)	a	Neg.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	2,4
2034824	Infant formula milk powder with probiotics (*Lactobacillus reuteri* 5,5.10^6 CFU/g)	a	Pos.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	2,6
2034827	Infant formula milk powder with probiotics (*Bifidobacterium lactis* 4,5.10^6 CFU/g)	a	Pos.	*Cronobacter sakazakii*	JCT201	Lyophilized	2 weeks at ambient temperature	2,8
2034828	Infant formula milk powder with probiotics (*Bifidobacterium lactis* 2,1.10^7 CFU/g)	a	Pos.	*Cronobacter sakazakii*	JCT201	Lyophilized	2 weeks at ambient temperature	2,8
2034845	Infant cereals with probiotics (cocoa) Batch 2 (*B. lactis* 5,6.10^6 CFU/g)	a	Pos.	*Cronobacter sakazakii*	AWX553	Air-dried	2 weeks at ambient temperature	2,2
2034884	Infant formula milk powder with probiotics (*Lactobacillus reuteri* 6,1.10^6 CFU/g)	a	Pos.	*Cronobacter sakazakii*	JGG241	Lyophilized	2 weeks at ambient temperature	2,4
2034885	Infant formula milk powder with probiotics (*Bifidobacterium infantis* 3,1.10^6 CFU/g)	a	Pos.	*Cronobacter sakazakii*	JGG241	Lyophilized	2 weeks at ambient temperature	2,4
2034886	Infant cereals with probiotics (honey) (*B. lactis* 3,4.10^6 CFU/g)	a	Pos.	*Cronobacter malonaticus*	HSE835	Air-dried	2 weeks at ambient temperature	1,2
2034892	Infant cereals with probiotics (*B. lactis* 6,8.10^6 CFU/g)	a	Pos.	*Cronobacter sakazakii*	AWX553	Air-dried	2 weeks at ambient temperature	1,0
2066812	Infant cereals with probiotics (chocolate & biscuit) (*B. lactis* 7,0.10^5 CFU/g)	a	Pos.	*Cronobacter turicensis*	HZN090	Air-dried	2 weeks at ambient temperature	2,8
2034830	Infant formula milk powder 6 - 12 months	b	Neg.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	2,6
2034831	Infant formula milk powder 0 - 6 months	b	Neg.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	2,6
2034832	Infant formula milk powder 12 - 36 months batch 1	b	Neg.	*Cronobacter malonaticus*	HSE835	Air-dried	2 weeks at ambient temperature	1,8
2034833	Infant formula milk powder 12 - 36 months batch 2	b	Neg.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	1,6
2034834	Infant cereals w/o probiotics	b	Neg.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	1,6
2034835	Infant formula milk powder 0 - 6 months	b	Neg.	*Cronobacter turicensis*	HZN090	Lyophilized	2 weeks at ambient temperature	2,6
2034848	Infant formula milk powder 12 - 36 months batch 2	b	Neg.	*Cronobacter turicensis*	HZN090	Lyophilized	2 weeks at ambient temperature	2,6
2034849	Infant cereals w/o probiotics (biscuit)	b	Neg.	*Cronobacter sakazakii*	JGG241	Lyophilized	2 weeks at ambient temperature	2,2
2034850	Infant cereals w/o probiotics (wheat & vanilla)	b	Neg.	*Cronobacter sakazakii*	JGG241	Lyophilized	2 weeks at ambient temperature	2,2
2034852	Infant cereals w/o probiotics (wheat & cocoa)	b	Pos.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	2,4
2066815	Infant formula milk powder 0 - 6 months	b	Pos.	*Cronobacter sakazakii*	HYD216	Lyophilized	2 weeks at ambient temperature	2,0
1978477	Infant cereals w/o probiotics	b	Pos.	*Cronobacter sakazakii*	AWX553	Air-dried	2 weeks at ambient temperature	2,2
2034851	Infant cereals w/o probiotics (vanilla)	b	Pos.	*Cronobacter turicensis*	HZN090	Air-dried	2 weeks at ambient temperature	2,0
2034888	Infant formula milk powder 6 - 12 months batch 1	b	Pos.	*Cronobacter malonaticus*	HSE835	Air-dried	2 weeks at ambient temperature	1,2
2034889	Infant formula milk powder 0 - 6 months	b	Pos.	*Cronobacter sakazakii*	HXN562	Lyophilized	2 weeks at ambient temperature	3,0
2034890	Infant formula milk powder 12 - 36 months batch 1	b	Pos.	*Cronobacter sakazakii*	HXN562	Lyophilized	2 weeks at ambient temperature	3,0
2034891	Infant formula milk powder 12 - 36 months batch 2	b	Pos.	*Cronobacter sakazakii*	JCT201	Lyophilized	2 weeks at ambient temperature	<1
2066813	Infant cereals w/o probiotics (wheat & cocoa)	b	Pos.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	3,0
2066814	Infant cereals w/o probiotics (biscuit)	b	Pos.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	3,2
2034836	Caseinate powder	c	Neg.	*Cronobacter sakazakii*	HXN562	Lyophilized	2 weeks at ambient temperature	1,2
2034837	Lactosérum powder	c	Neg.	*Cronobacter turicensis*	HZN090	Lyophilized	2 weeks at ambient temperature	2,6

APPENDIX C - Artificial contamination

Sample N°	Product (probiotics enumeration CFU/g)	Type	Result	Strain	Strain code	Seeding protocol		Contamination level (CFU/test portion)
2034839	Lecithine soja powder	c	Neg.	*Cronobacter sakazakii*	AWX553	Air-dried	2 weeks at ambient temperature	2,2
2034840	Lactose powder	c	Neg.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	2,6
1978476	Lactoserum permeat batch 2	c	Neg.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	2,4
1978478	Lactoserum permeat batch 1	c	Neg.	*Cronobacter sakazakii*	JGG241	Lyophilized	2 weeks at ambient temperature	2,2
2034838	Maltodextrine batch 1	c	Pos.	*Cronobacter malonaticus*	HSE835	Air-dried	2 weeks at ambient temperature	1,8
1978479	Rice flour batch 1	c	Pos.	*Cronobacter sakazakii*	AWX553	Air-dried	2 weeks at ambient temperature	2,2
1978480	Powdered whole milk	c	Pos.	*Cronobacter dublinensis*	GVV828	Lyophilized	2 weeks at ambient temperature	2,6
2034887	Lactoserum powder	c	Pos.	*Cronobacter sakazakii*	HXN562	Lyophilized	2 weeks at ambient temperature	3,0
2034893	Lactose	c	Pos.	*Cronobacter sakazakii*	HXN562	Lyophilized	2 weeks at ambient temperature	3,0
2034894	Lecithine soja powder	c	Pos.	*Cronobacter sakazakii*	HXN562	Lyophilized	2 weeks at ambient temperature	3,0
2034895	Powdered whole milk	c	Pos.	*Cronobacter sakazakii*	JCT201	Lyophilized	2 weeks at ambient temperature	<1
2066816	Lactoserum permeat	c	Pos.	*Cronobacter muytjensii*	GVW884	Air-dried	2 weeks at ambient temperature	3,2
2066817	Wheat flour	c	Pos.	*Cronobacter sakazakii*	HYD216	Lyophilized	2 weeks at ambient temperature	2,0

Appendix D - Sensitivity: raw data

Bold typing : artificially inoculated samples

***Cronobacter* detection results:**

m:	minoritary level of target analyte
M :	majoritary level of target analyte
P:	pure culture level of target analyte
1/2 :	50% level of target analyte
(x):	number of colonies in the plate
-:	no typical colonies but presence of background microflora
st:	plate without any colony
i:	PCR inhibition
PA:	positive agreement
NA:	negative agreement
ND:	negative deviation
PD:	positive deviation
PPNA:	positive presumptive negative agreement
PPND :	positive presumptive negative deviation
d:	doubtful result
NC:	non characteristic colony
ox:	oxidase test
*	1/5 dilution of the lysate
**	lysate stored at - 80°C tested again for this extension study

Discordant result between 7500 Fast (initial validation result) and QS5, lysates tested again with 7500 Fast in 2018 (**)

Lysate no more available

INFANT FORMULA MILK POWDER (10 g sample size)																												
Year of analysis	Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Reference method: ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																				Type
								BPW 16-20 h at 37°C ± 1°C										Storage 72 h for 5°C ± 3°C										
				CCI	Confirmatory tests		Final result	PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement			
					OX	Gallery		Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	
2016	**4885**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4886**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4887**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4888**	**Poudre de lait infantile 0-36 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4889**	**Poudre de lait infantile croissance**	**Infant formula milk powder**	+p	-	+	+	+(22,97)	+(23,61)	+(26,06)	+	+	+	+	**PA**	**PA**	**PA**	+(25,41)	+(21,28)	+(25,81)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**4890**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4891**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(25,65)	+(23,98)	+(28,14)	+	+	+	+	**PA**	**PA**	**PA**	+(23,08)	+(22,64)	+(24,72)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**4892**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	+p	-	+	+	+(21,34)	+(19,71)	+(23,55)	+	+	+	+	**PA**	**PA**	**PA**	+(20,57)	+(18,90)	+(22,88)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**4893**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(23,73)	+(23,78)	+(25,54)	+	+	+	+	**PA**	**PA**	**PA**	+(22,64)	+(22,47)	+(25,27)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**4894**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(27,04)	+(26,28)	+(34,09)	+	+	+	+	**PA**	**PA**	**PA**	+(26,13)	+(23,95)	+(28,90)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**4895**	**Poudre de lait infantile croissance**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4896**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4897**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4898**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4899**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**5070**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(25,75)	+(26,13)	+(28,96)	+	+	+	+	**PA**	**PA**	**PA**	+(26,02)	+(25,01)	+(26,11)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5071**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	+p	-	+	+	+(22,82)	+(22,46)	+(26,42)	+	+	+	+	**PA**	**PA**	**PA**	+(23,26)	+(21,25)	+(26,31)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5072**	**Poudre de lait infantile 1-3 ans**	**Infant formula milk powder**	+p	-	+	+	+(21,47)	+(20,40)	+(22,10)	+	+	+	+	**PA**	**PA**	**PA**	+(19,39)	+(18,35)	+(21,65)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5073**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	+p	-	+	+	+(21,46)	+(20,14)	+(21,73)	+	+	+	+	**PA**	**PA**	**PA**	+(21,16)	+(20,01)	+(21,87)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5074**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(22,78)	+(21,37)	+(21,95)	+	+	+	+	**PA**	**PA**	**PA**	+(22,84)	+(20,93)	+(23,67)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5075**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**5076**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(24,05)	+(23,40)	+(24,50)	+	+	+	+	**PA**	**PA**	**PA**	+(24,82)	+(23,72)	+(25,68)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5077**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	**5078**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	+p	-	+	+	+(23,97)	+(22,99)	+(24,07)	+	+	+	+	**PA**	**PA**	**PA**	+(24,03)	+(23,19)	+(25,23)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5079**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(26,83)	+(27,22)	+(27,68)	+	+	+	+	**PA**	**PA**	**PA**	+(26,37)	+(25,56)	+(26,76)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5080**	**Poudre de lait infantile 0-6 mois**	**Infant formula milk powder**	+p	-	+	+	+(25,86)	+(24,18)	+(26,64)	+	+	+	+	**PA**	**PA**	**PA**	+(24,84)	+(22,84)	+(26,22)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**5081**	**Poudre de lait infantile 6-12 mois**	**Infant formula milk powder**	+p	-	+	+	+(23,37)	+(21,80)	+(25,00)	+	+	+	+	**PA**	**PA**	**PA**	+(22,40)	+(20,92)	+(22,80)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	5203	Poudre de lait infantile-Bio	Infant formula milk powder	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a

INFANT FORMULA MILK POWDER (10 g sample size)																												
Year of analysis	Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Reference method: ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																				Type
								BPW 16-20 h at 37°C ± 1°C										Storage 72 h for 5°C ± 3°C										
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement			
				CCI	OX	Gallery	Final result	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	
2016	5204	Poudrede lait infantile-Croissance	Infant formula milk powder	st			-	-	-	-	-	-	-	-	NA	NA	NA											a
2016	5205	Poudrede lait infantile-Croissance	Infant formula milk powder	st			-	-	-	-	-	-	-	-	NA	NA	NA											a
2016	**1455**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,0.10^4/g)**	+	-	+	+	+(25,95)	+(27,75)	+(27,35)	+	+	+	+	PA	PA	PA											b
2016	**1456**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (8,0.10^4/g)**	+	-	+	+	+(24,89)	+(22,21)	+(24,74)	+	+	+	+	PA	PA	PA											b
2016	**1457**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,6.10^6/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1458**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,0.10^5/g)**	+	-	+	+	+(36,80)	+(38,42)	+(37,80)	+	+	+	+	PA	PA	PA											b
2016	**1459**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (5,8.10^7/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1460**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	+	-	+	+	+(32,84)	+(33,80)	+(32,93)	+	+	+	+	PA	PA	PA											b
2016	**1461**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	+	-	+	+	+(32,30)	+(34,44)	+(33,57)	+	+	+	+	PA	PA	PA											b
2016	**1462**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1463**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1464**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<2,0.10^4/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1465**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,2.10^6/g)**	+	-	+	+	+(32,89)	+(32,56)	+(32,66)	+	+	+	+	PA	PA	PA											b
2016	**1466**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,3.10^7/g)**	+	-	+	+	+(26,03)	+(22,22)	+(26,92)	+	+	+	+	PA	PA	PA											b
2016	**1467**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,5.10^6/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1468**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^4/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**1469**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,2.10^7/g)**	st			-	-	i/-	-	-	-	-	-	NA	NA	NA											b
2016	**2303**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,7.10^6/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**2304**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (5,4.10^6/g)**	+	-	+	+	+(30,57)	+(27,53)	i/+(28,41)*	+	+	+	+	PA	PA	PA											b
2016	**2305**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (6,0.10^6/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA											b

INFANT FORMULA MILK POWDER (10 g sample size)																												
Year of analysis	Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Reference method: ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																				Type
								BPW 16-20 h at 37°C ± 1°C										Storage 72 h for 5°C ± 3°C										
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement			
				CCI	OX	Gallery	Final result	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	
2016	**2306**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (7,0.10^{5}/g)**	st			-	+(30,12)	+(28,61)	+(29,97)	+	+	+	+	**PD**	**PD**	**PD**											b
2016	**2307**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (8,0.10^{5}/g)**	+	-	+	+	+(24,11)	+(20,75)	+(23,33)	+	+	+	+	**PA**	**PA**	**PA**											b
2016	**2308**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (3,2.10^{5}/g)**	+	-	+	+	+(24,22)	+(21,62)	+(23,86)	+	+	+	+	**PA**	**PA**	**PA**											b
2016	**2309**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (8,8.10^{5}/g)**	+	-	+	+	+(25,04)	+(22,75)	+(24,80)	+	+	+	+	**PA**	**PA**	**PA**											b
2016	**2310**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (3,6.10^{6}/g)**	st			-	i/-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	**4900**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^{4}/g)**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	/				/						b
2016	**4901**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,8.10^{4}/g)**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	/				/						b
2016	**4902**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (<200/g)**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	/				/						b
2016	**4903**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^{6}/g)**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	/				/						b
2016	**5082**	**Poudre de lait infantile avec probiotiques-croissance**	**Infant formula milk powder with probiotics (<200/g)**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	/				/						b
2016	**5206**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (1,4.10^{3}/g)**	st			-	-	+(38,64)	+(40,10)	-	-	-	-	**NA**	**PPNA**	**PPNA**	/				/						b
2016	**5207**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,0.10^{2}/g)**	st			-	+(42,00)	-	-	-	-	-	-	**PPNA**	**NA**	**NA**	/				/						b
2016	**5208**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^{2}/g)**	st			-	+(41,52)	+(39,00)	+(39,34)	-	-	-	-	**PPNA**	**PPNA**	**PPNA**	/				/						b
2016	**5240**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (7,9.10^{6}/g)**	+p	-	+	+	+(24,85)	+(22,94)	+(24,45)	+	+	+	+	**PA**	**PA**	**PA**	/				/						b
2016	**5241**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (4,0.10^{4}/g)**	+p	-	+	+	+(22,47)	+(20,54)	+(22,02)	+	+	+	+	**PA**	**PA**	**PA**	/				/						b
2016	**5242**	**Poudre de lait infantile avec probiotiques**	**Infant formula milk powder with probiotics (2,0.10^{4}/g)**	+	-	+	+	+(25,17)	+(22,68)	+(25,78)	+	+	+	+	**PA**	**PA**	**PA**	/				/						b

PRODUCTION ENVIRONMENTAL SAMPLES																												
Year of analysis	Sample N°	Product (French name)	Product	Reference method : ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																				Type
								BPW + 6 mg/L vancomycin 18-22 h at 37°C ± 1°C																				
																	Storage for 72 h at 5°C ± 3°C											
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement			
				CCI	OX	Gallery	Final result	PikoReal	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	
2016	**2049**	**Eau de rinçage**	**Rinsing water**	+	-	+	+	+(21,40)	+(19,08)	+(24,99)	+	+	+	+	**PA**	**PA**	**PA**	+(21,15)	+(18,95)	+(22,33)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**2050**	**Eau de rinçage**	**Rinsing water**	+	-	+	+	+(21,67)	+(20,01)	+(22,76)	+	+	+	+	**PA**	**PA**	**PA**	+(21,73)	+(19,16)	+(22,53)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**2051**	**Eau de rinçage**	**Rinsing water**	+	-	+	+	+(20,59)	+(18,58)	+(24,58)	+	+	+	+	**PA**	**PA**	**PA**	+(21,25)	+(19,83)	+(20,75)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**2052**	**Eau de rinçage**	**Rinsing water**	+	-	+	+	+(21,15)	+(20,16)	i/+(24,97)*	+	+	+	+	**PA**	**PA**	**PA**	+(22,64)	+(21,25)	+(24,17)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**2053**	**Eau de rinçage**	**Rinsing water**	st			-	+(21,12)	+(19,58)	+(21,85)	+	+	+	+	**PD**	**PD**	**PD**	+(20,54)	+(19,58)	+(22,18)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**2446**	**Eau de process**	**Process water**	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	**2447**	**Eau de nettoyage**	**Cleaning water**	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	4075	Eau de rinçage	Rinsing water	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	4076	Eau de rinçage	Rinsing water	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	4077	Eau de rinçage	Rinsing water	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	4078	Eau de rinçage	Rinsing water	st			-	-	-	i/-*	-	-	-	-	**NA**	**NA**	**NA**											a
2016	4079	Eau de rinçage	Rinsing water	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**4085**	**Eau de rinçage**	**Rinsing water**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	**4086**	**Eau de rinçage**	**Rinsing water**	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	**4087**	**Eau de rinçage**	**Rinsing water**	st			-	+(42,36)	-	-	-	-	-	-	**PPNA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	**4398**	**Eau de rinçage production PDL**	**Rinsing water**	+	-	+	+	+(20,86)	+(19,56)		+	+	+		**PA**	**PA**		+(22,95)	+(22,06)		+	+	+		**PA**	**PA**		a
2016	**4399**	**Eau de rinçage FOARMOR**	**Rinsing water**	+	-	+	+	+(30,64)	+(28,83)		+	+	+		**PA**	**PA**		+(33,71)	+(29,88)		+(1)	+	+		**PA**	**PA**		a
2016	4024	Eau de process pâtisserie	Process water (pastry)	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	4027	Eau de rinçage	Rinsing water	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	4028	Eau de process	Process water	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											5
2016	**2448**	**Eau de syphon**	**Siphon water**	+	-	+	+	+(26,13)	+(23,27)	+(26,15)	+	+	+	+	**PA**	**PA**	**PA**	+(25,81)	+(22,66)	+(26,52)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**2677**	**Poussièrrs laiterie**	**Dusts**	+	-	+	+	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	b
2016	**2678**	**Poussières aspirateur**	**Dusts**	+	-	+	+	+(25,69)	+(23,23)	+(24,47)	+	+	+	+	**PA**	**PA**	**PA**	+(24,19)	+(23,15)	+(25,93)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**2679**	**Déchets poudre de lait**	**Dusts**	+	-	+	+	+(29,60)	+(27,21)	+(27,80)	+	+	+	+	**PA**	**PA**	**PA**	+(28,43)	+(26,56)	+(28,54)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**2680**	**Poussières laiterie**	**Dusts**	+	-	+	+	+(26,85)	+(24,60)	+(25,80)	+	+	+	+	**PA**	**PA**	**PA**	+(25,14)	+(23,54)	+(24,63)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**2681**	**Poussières aspirateur**	**Dusts**	+	-	+	+	+(25,57)	+(22,81)	+(23,87)	+	+	+	+	**PA**	**PA**	**PA**	+(24,85)	+(22,29)	+(23,12)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**2682**	**Déchets poudre de lait**	**Dusts**	-			-	+(31,71)	+(29,48)	+(30,85)	+	+	+	+	**PD**	**PD**	**PD**	+(32,2)	+(29,08)	+(30,67)	+	+	+	+	**PD**	**PD**	**PD**	b
2016	**3479**	**Eponge poussières (industrie PDL)**	**Dusts sponge**	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	**3480**	**Eponge poussières (industrie PDL)**	**Dusts sponge**	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	**3481**	**Eponge poussières (industrie PDL)**	**Dusts sponge**	+	-	+	+	+(31,60)	+(30,99)	+(32,94)	+	+	+	+	**PA**	**PA**	**PA**	+(32,10)	+(28,11)	+(30,92)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	3488	Eponge poussières (industrie PDL)	Dusts sponge	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	3489	Eponge poussières (industrie PDL)	Dusts sponge	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	3490	Eponge poussières (industrie PDL)	Dusts sponge	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	b
2016	3491	Eponge poussières (industrie PDL)	Dusts sponge	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	3492	Eponge poussières (industrie PDL)	Dusts sponge	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											b
2016	3493	Eponge poussières (industrie PDL)	Dusts sponge	-			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**								**NA**	**NA**	**NA**	b

PRODUCTION ENVIRONMENTAL SAMPLES																												
Year of analysis	Sample N°	Product (French name)	Product	Reference method : ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																				Type
								BPW + 6 mg/L vancomycin 18-22 h at 37°C ± 1°C												Storage for 72 h at 5°C ± 3°C								
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement			
				CCI	OX	Gallery	Final result	PikoReal	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	
2016	3494	Eponge poussières (industrie PDL)	Dusts sponge	-			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	3495	Eponge poussières (industrie PDL)	Dusts sponge	-			-	-	-/-**	+(40,85)	-	-	-	-	NA	NA	PPNA											b
2016	4025	Chiffonnette poussière PDL	Wipe	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	4026	Chiffonnette déchets	Wipe	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**2055**	**Lingette étagère salle de stockage poudre de lait**	**Wipe**	+	-	+	+	+(21,03)	+(19,50)	+(22,53)	+	+	+	+	PA	PA	PA	+(23,41)	+(19,42)	+(22,54)	+	+	+	+	PA	PA	PA	c
2016	**2056**	**Lingette étagère salle de stockage poudre de lait**	**Wipe**	+	-	+	+	+(21,06)	+(19,35)	+(20,97)	+	+	+	+	PA	PA	PA	+(21,34)	+(18,09)	+(21,80)	+	+	+	+	PA	PA	PA	c
2016	**2057**	**Lingette plan de travail salle de stockage poudre de lait**	**Wipe**	+	-	+	+	+(21,95)	+(19,88)	+(24,34)	+	+	+	+	PA	PA	PA	+(22,65)	+(20,77)	+(23,62)	+	+	+	+	PA	PA	PA	c
2016	**2058**	**Lingette plan de travail salle de stockage poudre de lait**	**Wipe**	st			-	+(21,54)	+(19,48)	+(21,85)	+	+	+	+	PD	PD	PD	+(21,68)	+(18,96)	+(22,40)	+	+	+	+	PD	PD	PD	c
2016	**2440**	**Lingette transport pneumatique avant nettoyage**	**Wipe**	-			-	+(31,27)	+(28,64)	+(31,29)	+	+	+	+	PD	PD	PD	+(23,86)	+(23,16)	+(26,44)	+	+	+	+	PD	PD	PD	c
2016	**2441**	**Lingette sol**	**Wipe**	+	-	+	+	+(28,50)	+(25,88)	+(27,19)	+	+	+	+	PA	PA	PA	+(27,37)	+(25,24)	+(28,58)	+	+	+	+	PA	PA	PA	c
2016	**2443**	**Lingette transport pneumatique après nettoyage**	**Wipe**	+	-	+	+	+(21,67)	+(19,40)	+(20,49)	+	+	+	+	PA	PA	PA	+(21,11)	+(19,38)	+(22,00)	+	+	+	+	PA	PA	PA	c
2016	**2444**	**Lingette sol après nettoyage**	**Wipe**	+	-	+	+	+(27,40)	+(24,34)	+(27,40)	+	+	+	+	PA	PA	PA	+(27,87)	+(24,77)	+(28,99)	+	+	+	+	PA	PA	PA	c
2016	**2445**	**Lingette porte tour séchage après nettoyage**	**Wipe**	+	-	+	+	+(42,36)/+ (47,63)	-	-	-	-	-	-	PPND	ND	ND	-	-	-	-	-	-	-	ND	ND	ND	c
2016	**3339**	**Ecouvillon douchette réserve**	**Swab**	+	-	+	+	+(20,97)	+(19,61)	+(20,77)	+	+	+	+	PA	PA	PA	+(20,49)	+(19,51)	+(20,87)	+	+	+	+	PA	PA	PA	c
2016	**3340**	**Ecouvillon dilumate**	**Swab**	+	-	+	+	+(21,53)	+(20,35)	+(21,89)	+	+	+	+	PA	PA	PA	+(20,63)	+(19,26)	+(20,96)	+	+	+	+	PA	PA	PA	c
2016	**3341**	**Ecouvillon E5**	**Swab**	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	**3342**	**Ecouvillon 35**	**Swab**	+	-	+	+	+(24,34)	+(20,12)	+(22,70)	+	+	+	+	PA	PA	PA	+(21,26)	+(20,21)	+(20,70)	+	+	+	+	PA	PA	PA	c
2016	3343	Ecouvillon E6	Swab	-			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	3344	Ecouvillon 38	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	3345	Ecouvillon 41	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	**3477**	**Eponge surface (industrie PDL)**	**Surface sponge**	+	-	+	+	-	-	-	-	-	-	-	ND	ND	ND	-	-	-	-	-	-	-	ND	ND	ND	c
2016	**3478**	**Eponge surface (industrie PDL)**	**Surface sponge**	+	-	+	+	+(25,07)	+(23,40)	+(25,93)	+	+	+	+	PA	PA	PA	+(25,27)	+(22,20)	+(26,15)	+	+	+	+	PA	PA	PA	c
2016	3482	Eponge surface (industrie PDL)	Surface sponge	st			-	+(38,57)/+ (39,26)	-	-	-	-	-	-	PPNA	NA	NA	+(37,45)	+(36,50)/ +(38,41**)	-	-	-	-	-	PPNA	PPNA	NA	c
2016	3483	Eponge surface (industrie PDL)	Surface sponge	+	-	+	+	+(29,59)	+(27,75)	+(31,56)	+	+	+	+	PA	PA	PA	+(30,08)	+(28,30)	+(30,27)	+	+	+	+	PA	PA	PA	c
2016	3484	Eponge surface (industrie PDL)	Surface sponge	+	-	+	+	+(26,77)	+(25,21)	+(26,04)	+	+	+	+	PA	PA	PA	+(26,90)	+(24,70)	+(28,31)	+	+	+	+	PA	PA	PA	c
2016	3485	Eponge surface (industrie PDL)	Surface sponge	st			-	-	-	-	-	-	-	-	NA	NA	NA	-	-	-	-	-	-	-	NA	NA	NA	c
2016	3486	Eponge surface (industrie PDL)	Surface sponge	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	3487	Eponge surface (industrie PDL)	Surface sponge	-			-	-	-	-	-	-	-	-	NA	NA	NA	-	-	-	-	-	-	-	NA	NA	NA	c
2016	4080	Ecouvillon sol portoir	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	4081	Ecouvillon sol passerelle	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA	-	-	-	-	-	-	-	NA	NA	NA	c
2016	4082	Ecouvillon sol circulation	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	4083	Ecouvillon mélangeur	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA											c
2016	4084	Ecouvillon SAS	Swab	st			-	-	-	-	-	-	-	-	NA	NA	NA											c

INFANT FORMULA (300 g sample size)																													
Year of analysis	Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Reference method : ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																					Type
								BPW + 6 mg/L vancomycin 18-22 h at 37°C ± 1°C										Storage for 72 h at 5°C ± 3°C											
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement				
				CCI	OX	Gallery	Final result	PikoReal	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		
2016	**3265**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	+p	-	+	+	+(19,18)	+(19,42)	+(20,50)	+	+	+	+	**PA**	**PA**	**PA**	+(18,29)	+(19,35)	+(20,86)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3266**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(21,16)	+(21,29)	+(22,14)	+	+	+	+	**PA**	**PA**	**PA**	+(19,49)	+(19,43)	+(22,76)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3267**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	+(41,05)/-/+(42,43)	-/+(39,68**)	+(46,58)	-	-	-	-	**PPNA**	**NA**	**PPNA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	a
2016	**3268**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	-	-/-**	+(44,15)	-	-	-	-	**ND**	**ND**	**PPND**	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	a
2016	**3269**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	+(28,06)	+(27,26)	+(30,05)	+	+	+	+	**PD**	**PD**	**PD**	+(24,81)	+(25,22)	+(27,34)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3270**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	+p	-	+	+	+(30,18)	+(29,53)	+(34,84)	+	+	+	+	**PA**	**PA**	**PA**	+(29,11)	+(29,24)	+(30,16)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3271**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	a
2016	**3272**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	+(32,93)	+(33,00)	+(36,12)	+	+	+	+	**PD**	**PD**	**PD**	+(27,82)	+(28,29)	+(29,95)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3273**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	+(30,72)	+(30,90)	+(33,02)	+	+	+	+	**PD**	**PD**	**PD**	+(25,24)	+(26,11)	+(34,86)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	3363	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3365	Poudre de lait infantile 1er âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3367	Poudre de lait infantile 1er âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3368	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3369	Poudre de lait infantile 1er âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3370	Poudre de lait infantile 1er âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3371	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3372	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	i/-*	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3374	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3375	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3376	Poudre de lait infantile 1er âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	3377	Poudre de lait infantile 2e âge	Infant formula without probiotics	st			-	-	-	-	-	-	-	-	**NA**	**NA**	**NA**											a
2016	**3483**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	+(26,70)	+(26,64)	+(25,77)	+	+	+	+	**PD**	**PD**	**PD**	+(21,69)	+(22,67)	+(25,20)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3484**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3485**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3486**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-/-/-	-/-/-	-	+	-	-	-	**NA**	**NA**	**NA**	+(36,49)	+(35,74)	+(39,66)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3487**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-/-/-	-/-/-	-	+	-	-	-	**NA**	**NA**	**NA**	+(34,84)	+(36,61)	+(22,00)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3488**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3753**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3754**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	+(30,38)	+(31,20)	+(37,52)	+	+	+	+	**PD**	**PD**	**PD**	+(29,01)	+(29,30)	+(33,95)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3755**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3756**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3757**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3758**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	+(35,07)	+(34,23)	+(36,73)	+	+	+	+	**PD**	**PD**	**PD**	+(32,00)	+(32,33)	+(33,32)	+	+	+	+	**PD**	**PD**	**PD**	a
2016	**3759**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	-	-	-		-	-	-	**ND**	**ND**	**ND**	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	a
2016	**3760**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											a
2016	**3761**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	-	-	-		-	-	-	**ND**	**ND**	**ND**	-	-	-	-	-	-	-	**ND**	**ND**	**ND**	a
2016	**3940**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(20,32)	+(19,87)	+(21,28)	+	+	+	+	**PA**	**PA**	**PA**	+(18,35)	+(18,35)	+(19,35)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3941**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(20,15)	+(19,47)	+(21,31)	+	+	+	+	**PA**	**PA**	**PA**	+(18,31)	+(17,42)	+(19,99)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3942**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(19,43)	+(19,21)	+(22,28)	+	+	+	+	**PA**	**PA**	**PA**	+(19,15)	+(19,38)	+(20,45)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3943**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(21,2)	+(21,92)	+(25,35)	+	+	+	+	**PA**	**PA**	**PA**	+(19,24)	+(19,38)	+(20,99)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3944**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(20,28)	+(19,25)	+(23,62)	+	+	+	+	**PA**	**PA**	**PA**	+(21,48)	+(20,65)	+(21,11)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3945**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	+p	-	+	+	+(20,12)	+(19,39)	+(23,22)	+	+	+	+	**PA**	**PA**	**PA**	+(20,69)	+(19,71)	+(21,04)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3946**	**Poudre de lait infantile 1er âge**	**Infant formula without probiotics**	+p	-	+	+	+(20,51)	+(19,20)	+(24,13)	+	+	+	+	**PA**	**PA**	**PA**	+(19,00)	+(18,95)	+(21,91)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**3947**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	st			-	-	-	-	+	-	-	-	**NA**	**NA**	**NA**	i/-	-/+(37,02**)	+(38,35)	+	-	-	+	**NA**	**NA**	**PD**	a
2016	**3948**	**Poudre de lait infantile 2e âge**	**Infant formula without probiotics**	+p	-	+	+	+(20,6)	+(20,02)	+(23,18)	+	+	+	+	**PA**	**PA**	**PA**	+(18,20)	+(17,64)	+(20,21)	+	+	+	+	**PA**	**PA**	**PA**	a
2016	**1750**	**Poudre de lait infantile 1er âge avec probiotiques (<10cfu/g)**	**Infant formula with probiotics (<10cfu/g)**	+p	-	+	+	+(19,97)	+(19,03)	+(20,24)	+	+	+	+	**PA**	**PA**	**PA**	+(18,85)	+(17,75)	+(19,11)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**1751**	**Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)**	**Infant formula with probiotics (<10cfu/g)**	+p	-	+	+	+(21,37)	+(20,99)	+(19,90)	+	+	+	+	**PA**	**PA**	**PA**	+(19,33)	+(18,05)	+(19,67)	+	+	+	+	**PA**	**PA**	**PA**	b
2016	**1752**	**Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)**	**Infant formula with probiotics (<10cfu/g)**	+p	-	+	+	+(21,65)	+(20,69)	+(21,68)	+	+	+	+	**PA**	**PA**	**PA**	+(19,78)	+(18,35)	+(20,21)	+	+	+	+	**PA**	**PA**	**PA**	b

INFANT FORMULA (300 g sample size)																													
Year of analysis	Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Reference method : ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																					Type
								BPW + 6 mg/L vancomycin 18-22 h at 37°C ± 1°C										Storage for 72 h at 5°C ± 3°C											
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement				
				CCI	OX	Gallery	Final result	PikoReal	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		
2016	**1753**	**Poudre de lait infantile 1er âge avec probiotiques (1,5 10^5 ufc/g)**	**Infant formula with probiotics (1,5 10^5 cfu/g)**	+p	-	+	+	+(19,87)	+(18,59)	+(24,08)	+	+	+	+	PA	PA	PA	+(20,15)	+(18,70)	+(20,40)	+	+	+	+	PA	PA	PA	b
2016	**1754**	**Poudre de lait infantile 1er âge avec probiotiques (9,7 10^5 ufc/g)**	**Infant formula with probiotics (9,7 10^5 cfu/g)**	+p	-	+	+	+(20,24)	+(19,19)	+(23,03)	+	+	+	+	PA	PA	PA	+(20,75)	+(20,18)	+(21,08)	+	+	+	+	PA	PA	PA	b
2016	**1755**	**Poudre de lait infantile 3e âge avec probiotiques (8,4 10^4 ufc/g)**	**Infant formula with probiotics (8,4 10^4 cfu/g)**	+p	-	+	+	-	-/+(19,12**)	+(22,00)	-	-	-	-	ND	ND	PPND	-	-	-	-	-	-	-	ND	ND	ND	b
2016	**1756**	**Poudre de lait infantile 1er âge avec probiotiques (1,0 10^6 ufc/g)**	**Infant formula with probiotics (1,0 10^6 cfu/g)**	+p	-	+	+	+(22,38)	+(21,80)	+(24,16)	+	+	+	+	PA	PA	PA	+(21,15)	+(20,48)	+(21,09)	+	+	+	+	PA	PA	PA	b
2016	**1757**	**Poudre de lait infantile 1er âge avec probiotiques (2,2 10^5 ufc/g)**	**Infant formula with probiotics (2,2 10^5 cfu/g)**	st			-	+(28,02)	+(27,46)	+(29,80)	+	+	+	+	PD	PD	PD	+(22,41)	+(22,06)	+(23,31)	+	+	+	+	PD	PD	PD	b
2016	**1758**	**Poudre de lait infantile 2e âge avec probiotiques (1,1 10^6 ufc/g)**	**Infant formula with probiotics (1,1 10^6 cfu/g)**	+p	-	+	+	+(31,02)	+(30,22)	+(32,48)	+	+	+	+	PA	PA	PA	+(26,21)	+(24,43)	+(26,30)	+	+	+	+	PA	PA	PA	b
2016	**1759**	**Poudre de lait infantile 1er âge avec probiotiques (1,0 10^6 ufc/g)**	**Infant formula with probiotics (1,0 10^6 cfu/g)**	st			-	+(25,08)	+(24,00)	+(26,71)	+	+	+	+	PD	PD	PD	+(21,62)	+(20,35)	+(23,44)	+	+	+	+	PD	PD	PD	b
2016	**1760**	**Poudre de lait infantile 2e âge avec probiotiques (20 ufc/g)**	**Infant formula with probiotics (20 cfu/g)**	st			-	-	-	-	-	-	-	-	NA	NA	NA	-	-	-	-	-	-	-	NA	NA	NA	b
2016	**1761**	**Poudre de lait infantile 1er âge avec probiotiques (8,0 10^5 ufc/g)**	**Infant formula with probiotics (8,0 10^5 cfu/g)**	+p	-	+	+	+(20,67)	+(19,10)	+(21,93)	+	+	+	+	PA	PA	PA	+(20,42)	+(18,76)	+(21,52)	+	+	+	+	PA	PA	PA	b
2016	**1762**	**Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)**	**Infant formula with probiotics (<10cfu/g)**	+p	-	+	+	+(21,12)	+(20,32)	+(22,57)	+	+	+	+	PA	PA	PA	+(19,65)	+(18,58)	+(20,61)	+	+	+	+	PA	PA	PA	b
2016	**1763**	**Poudre de lait infantile 1er âge avec probiotiques (6,0 10^5 ufc/g)**	**Infant formula with probiotics (6,0 10^5 cfu/g)**	+p	-	+	+	+(24,98)	+(23,44)	+(26,78)	+	+	+	+	PA	PA	PA	+(22,54)	+(21,17)	+(23,57)	+	+	+	+	PA	PA	PA	b
2016	**1764**	**Poudre de lait infantile 1er âge avec probiotiques (3,0 105 ufc/g)**	**Infant formula with probiotics (3,0 10^5 cfu/g)**	+p	-	+	+	+(22,81)	+(22,07)	+(24,89)	+	+	+	+	PA	PA	PA	+(22,62)	+(21,62)	+(23,44)	+	+	+	+	PA	PA	PA	b
2016	**3264**	**Poudre de lait infantile 1er âge avec probiotiques (3,9 105 ufc/g)**	**Infant formula with probiotics (3,9 10^5 cfu/g)**	+p	-	+	+	+(20,68)	+(20,96)	+(22,12)	+	+	+	+	PA	PA	PA	+(19,70)	i/+(20,24)	+(21,92)	+	+	+	+	PA	PA	PA	b
2016	3364	Poudre de lait infantile 2e âge avec probiotiques (1,1 10^6 ufc/g)	Infant formula with probiotics (1,1 10^6 cfu/g)	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	3366	Poudre de lait infantile 1er âge avec probiotiques (8,0 10^5 ufc/g)	Infant formula with probiotics (8,0 10^5 cfu/g)	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	3373	Poudre de lait infantile 1er âge avec probiotiques (9,7 10^5 ufc/g)	Infant formula with probiotics (9,7 10^5 cfu/g)	st			-	-	-	-	-	-	-	-	NA	NA	NA											b
2016	**3489**	**Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)**	**Infant formula with probiotics (<10cfu/g)**	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	**3490**	**Poudre de lait infantile 2e âge avec probiotiques (1,1 106 ufc/g)**	**Infant formula with probiotics (1,1 10^6 cfu/g)**	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	3687	Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)	Infant formula with probiotics (<10cfu/g)	st			-	+(25,22)	+(25,51)	+(27,51)	+	+	+	+	PD	PD	PD	+(20,37)	+(20,62)	+(26,07)	+p	+	+	+	PD	PD	PD	b
2016	3688	Poudre de lait infantile 1er âge avec probiotiques (1,1 10^6 ufc/g)	Infant formula with probiotics (8,7 10^6 cfu/g)	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	3689	Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)	Infant formula with probiotics (<10cfu/g)	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	3690	Poudre de lait infantile 2e âge avec probiotiques (3,0 10^4 ufc/g)	Infant formula with probiotics (3,0 10^4 cfu/g)	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	3691	Poudre de lait infantile 1er âge avec probiotiques (10 ufc/g)	Infant formula with probiotics (10 cfu/g)	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	3692	Poudre de lait infantile 3ième âge avec probiotiques (5,1 10^2 ufc/g)	Infant formula with probiotics (5,1 10^2 cfu/g)	st			-	-	-	-		-	-	-	NA	NA	NA											b
2016	3693	Poudre de lait infantile 2e âge avec probiotiques (1,0 10^5 ufc/g)	Infant formula with probiotics (1,0 10^5 cfu/g)	st			-	-	-	-		-	-	-	NA	NA	NA											b

INFANT FORMULA (300 g sample size)																													
Year of analysis	Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Reference method : ISO 22964♦				Thermo Scientific SureTect *Cronobacter* species																					Type
								BPW + 6 mg/L vancomycin 18-22 h at 37°C ± 1°C										Storage for 72 h at 5°C ± 3°C											
					Confirmatory tests			PCR result (Cq)			All confir-matory tests	Final result			Agreement			PCR result (Cq)			All confir-matory tests	Final result			Agreement			
				CCI	OX	Gallery	Final result	PikoReal	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5		Piko Real	7500 Fast	QS5	Piko Real	7500 Fast	QS5	
2016	3694	Poudre de lait infantile 1er âge avec probiotiques (4,0 10^5 ufc/g)	Infant formula with probiotics (4,0 10^5 cfu/g)	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											b
2016	3695	Poudre de lait infantile 1er âge avec probiotiques (<10ufc/g)	Infant formula with probiotics (<10cfu/g)	st			-	+(40,83)	-	-		-	-	-	**PPNA**	**NA**	**NA**	-	-	-	-	-	-	-	**NA**	**NA**	**NA**	b
2016	3696	Poudre de lait infantile 2e âge avec probiotiques (4,0 10^6 ufc/g)	Infant formula with probiotics (4,0 10^6 cfu/g)	st			-	+(33,99)/ +/+	+(33,78)/ +/+	+(35,20)	5xmLST :st	-	-	-	**PPNA**	**PPNA**	**PPNA**	+(33,65)	+(33,33)	+(36,17)	-	-	-	-	**PPNA**	**PPNA**	**PPNA**	b
2016	3949	Poudre de lait infantile 3e âge avec probiotiques (<10ufc/g)	Infant formula with probiotics (<10cfu/g)	st			-	-	-	-		-	-	-	**NA**	**NA**	**NA**											b

APPENDIX D

Sensitivity raw results - Extension study (2021)

Bacterial burden
Ø: no culture
L = low
M = moderate
H = high
/: not realized

Distribution of flora
A = pure culture of suspect colonies
B = mixture with a majority of suspect colonies
C = mixture with a minority of suspect colonies
D = mixture with rare suspect colonies
E = absence of suspect colonies
(x): x colonies characteristic of Salmonella if x ≤ 5

PA: positive agreement
NA: negative agreement
ND: negative deviation
PD: positive deviation
PPNA: positive presumptive negative agreement
PPND : positive presumptive negative deviation
/: not realized

Type	Sample N°	Product (probiotics enumeration CFU/g)	Reference method (■)				Thermo Scientific SureTect *Cronobacter* species																				
			ISO 22964				BPW 6 mg/L vancomycine 20 h at 26 h at 34-38°C										Agreement		BPW 6 mg/L vancomycine After storage 72h at 4°C							Agreement	
			CCI after CSB	Confirmation tests		Final result	PCR result (Cq)		Confirmation tests: CCI+bioch			Confirmation tests: CSB+CCI+bioch			Final result				PCR result (Cq)		Confirmation tests: CCI+bioch			Final result			
				OX	Gallery		7500 Fast	QS5	CCI	OX	Gallery	CCI After CSB	OX	Gallery	7500 Fast	QS5	7500 Fast	QS5	7500 Fast	QS5	CCI	OX	Gallery	7500 Fast	QS5	7500 Fast	QS5
a-	2055439	Infant cereals with probiotics (milk chocolate) *B.lactis* 6,8.10^6 CFU/g	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	2034825	Infant formula milk powder with probiotics *Lactobacillus reuteri* 5,5.10^5 CFU/g	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	2034826	Infant formula milk powder with probiotics *Bifidobacterium infantis* 3,1.10^6 CFU/g	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	2034829	Infant formula milk powder with probiotics *Lactobacillus fermentum hereditum* 1,0.10^6 CFU/g	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	2034842	Infant formula milk powder with probiotics *Lactobacillus fermentum hereditum* 1,0.10^6 CFU/g	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	2034843	Infant cereals with probiotics (biscuit) *B. lactis* 7,0.10^5 CFU/g	Ø	/	/	A	-	-	EM	/	/	EM	/	/	A	A	NA	NA	-	-	EM	/	/	A	A	NA	NA
a-	2034844	Infant cereals with probiotics (honey) *B. lactis* 3,4.10^6 CFU/g	Ø	/	/	A	-	-	EL	/	/	EL	/	/	A	A	NA	NA	/	/	EL	/	/	A	A	NA	NA
a-	2034841	Infant cereals with probiotics *B. lactis* 6,8.10^6 CFU/g	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	1978475	Infant formula milk powder with probiotics *Lactobacillus reuteri* 4,0.10^5 CFU/g	Ø	/	/	A	-	+ (36,12) (-/-/-/-/-)	Ø	/	/	Ø	/	/	A	A (FP)	NA	NA (PP)	+ (37,14) -/-/-/ +(36,88)/+(36,48)	+ (36,01) -/-/ +(35,58) /+(35,11)/+(35,54)	Ø	/	/	A (FP)	A (FP)	NA (PP)	NA (PP)
a-	2034846	Infant cereals with probiotics (5 cereals) - *B. lactis* 5,0.10^5 CFU/g	Ø	/	/	A	+ (36,17) (-/-/-/-/-)	-	DL (1 colony~) CCI bis: -	-	/	Ø	/	/	A (FP)	A	NA (PP)	NA	+ (39,85) (-/-/-/-/-)	-	Ø	/	/	A (FP)	A	NA (PP)	NA
a-	2034847	Infant cereals with probiotics (chocolate & biscuit) - *B. lactis* 7,0.10^5 CFU/g	Ø	/	/	A	-	-	DL (1 colony~) CCI bis: -	-	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
a-	2045398	Infant cereals with probiotics (5 cereals) - *B. lactis* 5,0.10^5 CFU/g	Ø	/	/	A	-	-	EL	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
a+	2034824	Infant formula milk powder with probiotics *Lactobacillus reuteri* 5,5.10^6 CFU/g	AM	-	*Cronobacter*	P	-	-	Ø	/	/	Ø	/	/	A	A	ND	ND	-	-	EL	/	/	A	A	ND	ND
a+	2034827	Infant formula milk powder with probiotics *Bifidobacterium lactis* 4,5.10^6 CFU/g	AM	-	*Cronobacter*	P	-	-	Ø	/	/	Ø	/	/	A	A	ND	ND	-	-	Ø	/	/	A	A	ND	ND
a+	2034828	Infant formula milk powder with probiotics *Bifidobacterium lactis* 2,1.10^7 CFU/g	Ø	/	/	A	+ (19,68)	+ (19.12)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PD	PD	+ (19,32)	+ (17,80)	AM	-	*Cronobacter*	P	P	PD	PD
a+	2034845	Infant cereals with probiotics (cocoa) Batch 2 - *B. lactis* 5,6.10^6 CFU/g	Ø	/	/	A	(+ 20,29)	+ (19,47)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PD	PD	+ (23,74)	+ (22,68)	AM	-	*Cronobacter*	P	P	PD	PD
a+	2034884	Infant formula milk powder with probiotics *Lactobacillus reuteri* 6,1.10^6 CFU/g	AM	-	*Cronobacter*	P	+ (23,48)	+ (21,96)	AM	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PA	PA	+ (23,12)	+ (20,82)	AM	-	*Cronobacter*	P	P	PA	PA
a+	2034885	Infant formula milk powder with probiotics *Bifidobacterium infantis* 3,1.10^6 CFU/g	AM	-	*Cronobacter*	P	+ (22,08)	+ (19,86)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (20,46)	+ (19,67)	AM	-	*Cronobacter*	P	P	PA	PA
a+	2034886	Infant cereals with probiotics (honey) *B. lactis* 3,4.10^6 CFU/g	AM	-	*Cronobacter*	P	+ (26,80)	+ (24,87)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (20,17)	+ (18,84)	AM	-	*Cronobacter*	P	P	PA	PA
a+	2034892	Infant cereals with probiotics *B. lactis* 6,8.10^6 CFU/g	AL	-	*Cronobacter*	P	-	-	EL	/	/	EL	/	/	A	A	ND	ND	-	-	EL	/	/	A	A	ND	ND
a+	2045399	Infant cereals with probiotics (cocoa) Batch 1 - *B. lactis* 5,6.10^6 CFU/g	AM	-	*Cronobacter*	P	+ (25,81)	+ (22,95)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (25,84)	+ (24,38)	AM	-	*Cronobacter*	P	P	PA	PA
a+	2066812	Infant cereals with probiotics (chocolate & biscuit) - *B. lactis* 7,0.10^5 CFU/g	AM	-	*Cronobacter*	P	-	-	EL	/	/	Ø	/	/	A	A	ND	ND	-	-	EL	/	/	A	A	ND	ND
b-	2034830	Infant formula milk powder 6 - 12 months	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
b-	2034831	Infant formula milk powder 0 - 6 months	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
b-	2034832	Infant formula milk powder 12 - 36 months batch 1	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
b-	2034833	Infant formula milk powder 12 - 36 months batch 2	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
b-	2034834	Infant cereals w/o probiotics	Ø	/	/	A	-	-	CM blue & white colonies BIS: CCI -/-/-/-/-	-	ID32E: Ø Acineto-bacter baumanii	EM white colonies	/	/	A	A	NA	NA	-	-	EM white colonies	/	/	A	A	NA	NA
b-	2034835	Infant formula milk powder 0 - 6 months	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
b-	2034848	Infant formula milk powder 12 - 36 months batch 2	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA

Type	Sample N°	Product (probiotics enumeration CFU/g)	Reference method (■)				Thermo Scientific SureTect *Cronobacter* species																				
			ISO 22964				BPW 6 mg/L vancomycine 20 h at 26 h at 34-38°C										Agreement		BPW 6 mg/L vancomycine After storage 72h at 4°C							Agreement	
			CCI after CSB	Confirmation tests		Final result	PCR result (Cq)		Confirmation tests: CCI+bioch			Confirmation tests: CSB+CCI+bioch			Final result				PCR result (Cq)		Confirmation tests: CCI+bioch			Final result			
				OX	Gallery		7500 Fast	QS5	CCI	OX	Gallery	CCI After CSB	OX	Gallery	7500 Fast	QS5	7500 Fast	QS5	7500 Fast	QS5	CCI	OX	Gallery	7500 Fast	QS5	7500 Fast	QS5
b-	2034849	Infant cereals w/o probiotics (biscuit)	Ø	/	/	A	-	-	EL	/	/	Ø	/	/	A	A	NA	NA	/	/	EL	/	/	A	A	NA	NA
b-	2034850	Infant cereals w/o probiotics (wheat & vanilla)	EL	/	/	A	-	-	EL	/	/	Ø	/	/	A	A	NA	NA	/	/	EL	/	/	A	A	NA	NA
b-	2034852	Infant cereals w/o probiotics (wheat & cocoa)	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	+ (21,04)	+ (20,05)	AM	-	*Cronobacter*	P	P	PD	PD
b-	2045400	Infant formula milk powder w/o probiotics 0-6 months	Ø	/	/	A	-	-	EM	/	/	EL	/	/	A	A	NA	NA	-	-	EL	/	/	A	A	NA	NA
b-	2045401	Infant formula milk powder w/o probiotics 6-12 months	Ø	/	/	A	-	-	EM	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
b-	2045403	Infant formula milk powder w/o probiotics 6-12 months	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
b+	2066815	Infant formula milk powder 0 - 6 months	AM	-	*Cronobacter*	P	+ (23,45)	+ (22,18)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (22,57)	+ (24,71)	AM	-	*Cronobacter*	P	P	PA	PA
b+	1978477	Infant cereals w/o probiotics	AM	-	*Cronobacter*	P	+ (25,21)	+ (24,68)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (25,14)	+ (23,97)	BM	-	*Cronobacter*	P	P	PA	PA
b+	2034851	Infant cereals w/o probiotics (vanilla)	Ø	/	/	A	+ (20,40)	+ (18,84)	AM	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PD	PD	+ (20,07)	+ (18,63)	AM	-	*Cronobacter*	P	P	PD	PD
b+	2034888	Infant formula milk powder 6 - 12 months batch 1	Ø	/	/	A	+ (33,15)	+ (31,39)	AL	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PD	PD	+ (31,19)	+ (29,05)	AL	-	*Cronobacter*	P	P	PD	PD
b+	2034889	Infant formula milk powder 0 - 6 months	AM	-	*Cronobacter*	P	+ (20,29)	+ (19,62)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (20,74)	+ (18,82)	AM	-	*Cronobacter*	P	P	PA	PA
b+	2034890	Infant formula milk powder 12 - 36 months batch 1	AM	-	*Cronobacter*	P	+ (20,12)	+ (19,47)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (20,63)	+ (18,79)	AM	-	*Cronobacter*	P	P	PA	PA
b+	2034891	Infant formula milk powder 12 - 36 months batch 2	AL	-	*Cronobacter*	P	+ (19,64)	+ (19,61)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (19,77)	+ (17,54)	AL	-	*Cronobacter*	P	P	PA	PA
b+	2034853	Infant formula milk powder 6 - 12 months batch 2	Ø	/	/	A	+ (22,87)	+ (20,33)	AM	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PD	PD	+ 38,76 (5x+ 20,85/21,06/21,25/ 21,13/20,84)	+ 36,48 (5x+ 21,04/20,63/20,58/ 20,64/20,21)	AL	-	*Cronobacter*	P	P	PD	PD
b+	2066813	Infant cereals w/o probiotics (wheat & cocoa)	AL	-	*Cronobacter*	P	+ (19,64)	+ (19,54)	AM	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PA	PA	+ (19,20)	+ (19,66)	AM	-	*Cronobacter*	P	P	PA	PA
b+	2066814	Infant cereals w/o probiotics (biscuit)	AL	-	*Cronobacter*	P	+ (18,92)	+ (18,60)	AL	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PA	PA	+ (18,80)	+ (18,18)	AM	-	*Cronobacter*	P	P	PA	PA
c-	2034836	Caseinate powder	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
c-	2034837	Lactoserum powder	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
c-	2034839	Lecithin soja powder	Ø	/	/	A	-	-	EL	/	/	EL	/	/	A	A	NA	NA	/	/	Ø	/	/	A	A	NA	NA
c-	2034840	Lactose powder	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
c-	1978476	Lactoserum permeat batch 2	Ø	/	/	A	-	-	EM	/	/	EM white colonies	/	/	A	A	NA	NA	/	/	EM white colonies	/	/	A	A	NA	NA
c-	1978478	Lactoserum permeat batch 1	Ø	/	/	A	-	-	Ø	/	/	EL white colonies	/	/	A	A	NA	NA	/	/	EL white colonies	/	/	A	A	NA	NA
c-	2045402	Maltodextrin	Ø	/	/	A	-	-	EM	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
c-	2055440	Honey powder	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
c-	2055442	Powdered whole milk	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	-	-	Ø	/	/	A	A	NA	NA
c-	2055443	Wheat flour	Ø	/	/	A	+ (34,99) (-/-/34,20 /36,75/ 32,88)	-	Ø	/	/	Ø	/	/	A (FP)	A	NA (PP)	NA	-	34,35 (+/+/-/-/-)	EL	/	/	A	A (FP)	NA	NA (PP)
c-	2055444	Wheat starch	Ø	/	/	A	-	-	Ø	/	/	Ø	/	/	A	A	NA	NA	+ (38,34) (-/-/-/-/-)	-	Ø	/	/	A (FP)	A	NA (PP)	NA
c+	2034838	Maltodextrin batch 1	Ø	/	/	A	+ (20,38)	+ (19,44)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PD	PD	+ (22,25)	+ (20,20)	AM	-	*Cronobacter*	P	P	PD	PD
c+	1978479	Rice flour batch 1	AL	-	*Cronobacter*	P	+ (25,24)	+ (17,09)	AM	-	*Cronobacter*	BM	-	*Cronobacter*	P	P	PA	PA	+ (24,11)	+ (23,18)	BM	-	*Cronobacter*	P	P	PA	PA
c+	1978480	Powdered whole milk	AM	-	*Cronobacter*	P	+ (27,29)	+ (25,44)	AH	-	*Cronobacter*	AH	-	*Cronobacter*	P	P	PA	PA	+ (21,54)	+ (20,58)	AH	-	*Cronobacter*	P	P	PA	PA
c+	2034887	Lactoserum powder	AM	-	*Cronobacter*	P	+ (21,88)	+ (19,89)	AM	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PA	PA	+ (23,21)	+ (20,25)	AM	-	*Cronobacter*	P	P	PA	PA

Type	Sample N°	Product (probiotics enumeration CFU/g)	Reference method (■)				Thermo Scientific SureTect *Cronobacter* species																				
			ISO 22964				BPW 6 mg/L vancomycine 20 h at 26 h at 34-38°C										Agreement		BPW 6 mg/L vancomycine After storage 72h at 4°C							Agreement	
			CCI after CSB	Confirmation tests		Final result	PCR result (Cq)		Confirmation tests: CCI+bioch			Confirmation tests: CSB+CCI+bioch			Final result				PCR result (Cq)		Confirmation tests: CCI+bioch			Final result			
				OX	Gallery		7500 Fast	QS5	CCI	OX	Gallery	CCI After CSB	OX	Gallery	7500 Fast	QS5	7500 Fast	QS5	7500 Fast	QS5	CCI	OX	Gallery	7500 Fast	QS5	7500 Fast	QS5
c+	2034893	Lactose	AM	-	*Cronobacter*	P	+ (31,77)	+ (30,39)	AL	-	*Cronobacter*	AL	-	*Cronobacter*	P	P	PA	PA	+ (24,04)	+ (22,68)	AL	-	*Cronobacter*	P	P	PA	PA
c+	2034894	Lecithin soja powder	AM	-	*Cronobacter*	P	+ (27,80)	+ (26,59)	BH	-	*Cronobacter*	BM	-	*Cronobacter*	P	P	PA	PA	+ (26,34)	+ (25,50)	AM	-	*Cronobacter*	P	P	PA	PA
c+	2034895	Powdered whole milk	AM	-	*Cronobacter*	P	+ (30,71)	+ (28,77)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (24,88)	+ (24,03)	AM	-	*Cronobacter*	P	P	PA	PA
c+	2066816	Lactoserum permeat	AM	-	*Cronobacter*	P	+ (19,18)	+ (19,14)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (19,22)	+ (18,75)	AM	-	*Cronobacter*	P	P	PA	PA
c+	2066817	Wheat flour	AM	-	*Cronobacter*	P	+ (20,17)	+ (19,51)	AM	-	*Cronobacter*	AM	-	*Cronobacter*	P	P	PA	PA	+ (19,82)	+ (19,52)	AM	-	*Cronobacter*	P	P	PA	PA
c+	2055441	Rice flour batch 2	EM	/	/	A	+ (24,30)	+ (23,25)	BM	-	*Cronobacter*	BM	-	*Cronobacter*	P	P	PD	PD	+ (22,16)	+ (23,21)	BM	-	*Cronobacter*	P	P	PD	PD

Appendix E – Relative detection levels: raw data – Initial validation study

Infant formula with probiotics (10 g sample size)
Strain: *Cronobacter sakazakii* Ad 1418

Sample N°	Product	Inoculation level/ sample	Reference method : ISO 22964				Alternative method : SureTect *Cronobacter* species BPW-16 h at 37°C ± 1°C							
			CCI	Confirmatory tests	Final result	Number positive samples/ Total	PCR result (Cq value)		Confirmatory tests		Final result Piko Real	Final result 7500 Fast	Number positive samples/ Total PikoReal	Number positive samples/ Total 7500 Fast
							Piko Real	7500 Fast	CCI (direct streaking)	CCI (streaking after subculture in CSB)				
3627	Infant formula with probiotics (Anaerobic lactic flora 5,5 10^5 CFU/g)	0	st	/	-	0/5	-	-	st	st	-	-	0/5	0/5
3628			st	/	-		-	-	st	st	-	-		
3629			st	/	-		-	-	st	st	-	-		
3630			st	/	-		-	-	st	st	-	-		
3631			st	/	-		-	-	st	st	-	-		
3632		4,1	st	/	-	12/20	+(38,16)	+(38,21)	st	st	-	-	11/20	12/20
3633			+p	+	+		+(34,08)	+(30,86)	+p	+p	+	+		
3634			st	/	-		+(39,70)	+(34,85)	st	st	-	-		
3635			+p	+	+		+(41,64)	+(38,08)	+p	+p	+	+		
3636			+p	+	+		-	+(32,12)	+p	+p	-	+		
3637			+p	+	+		+(32,63)	+(29,87)	+p	+p	+	+		
3638			+p	+	+		+(42,39)	+(36,72)	+p	+p	+	+		
3639			+p	+	+		+(37,91)	+(34,07)	+p (2col)	+p	+	+		
3640			st	/	-		-	-	st	st	-	-		
3641			+p	+	+		+(38,53)	+(33,05)	+p	+p	+	+		
3642			+p	+	+		+(33,98)	+(29,29)	+p	+p	+	+		
3643			st	/	-		-	+(38,54)	st	st	-	-		
3644			st	/	-		-	-	st	st	-	-		
3645			+p	+	+		+(40,35)	+(34,32)	+p	+p	+	+		
3646			st	/	-		-	-	st	st	-	-		
3647			+p	+	+		+(36,53)	+(32,12)	+p	+p	+	+		
3648			st	/	-		-	-	st	st	-	-		
3649			+p	+	+		+(34,25)	+(31,16)	+p	+p	+	+		
3650			st	/	-		-	-	st	st	-	-		
3651			+p	+	+		+(39,11)	+(34,86)	+p	+p	+	+		
3652		8,2	+p	+	+	5/5	+(36,86)	+(37,77)	+p	+p	+	+	5/5	5/5
3653			+p	+	+		+(39,52)	+(36,98)	+p	+p	+	+		
3654			+p	+	+		+(40,10)	+(35,03)	+p	+p	+	+		
3655			+p	+	+		+(38,48)	+(35,22)	+p	+p	+	+		
3656			+p	+	+		+(36,85)	+(33,88)	+p	+p	+	+		

Infant formula without probiotics (10 g sample size)
Strain: Cronobacter sakazakii Ad 1418

Lysates discarded

Sample N°	Product	Inoculation level/sample	Reference method : ISO 22964				Alternative method : SureTect *Cronobacter* species BPW-16 h at 37°C ± 1°C							
			CCI	Confirmatory tests	Final result	Number positive samples/ Total	PCR result (Cq value)		Confirmatory tests		Final result Piko Real	Final result 7500 Fast	Number positive samples/ Total PikoReal	Number positive samples/ Total 7500 Fast
							Piko Real	7500 Fast	CCI (direct streaking)	CCI (streaking after subculture in CSB)				
4554	Infant formula (Aerobis mesophilic flora <10 CFU/g)	/	-	/	-	0/5	-	-	-	-	-	-	0/5	0/5
4555			-	/	-		-	-	-	-	-	-		
4556			-	/	-		-	-	-	-	-	-		
4557			-	/	-		-	-	-	-	-	-		
4558			-	/	-		-	-	-	-	-	-		
5050		3,5	+	+	+	15/20	+(24,77)	+(23,75)	+	+	+	+	15/20	15/20
5051			+	+	+		+(23,68)	+(23,22)	+	+	+	+		
5052			+	+	+		+(24,07)	+(23,34)	+	+	+	+		
5053			+	+	+		+(23,72)	+(23,27)	+	+	+	+		
5054			+	+	+		+(37,54)	+(35,39)	+	+	+	+		
5055			+	+	+		+(23,92)	+(22,60)	+	+	+	+		
5056			+	+	+		+(24,01)	+(23,04)	+	+	+	+		
5057			+	+	+		+(23,02)	+(22,03)	+	+	+	+		
5058			st	/	-		-	-	st	st	-	-		
5059			st	/	-		-	-	st	st	-	-		
5060			+	+	+		+(25,07)	+(24,73)	+	+	+	+		
5061			+	+	+		+(24,52)	+(22,49)	+	+	+	+		
5062			st	/	-		-	-	st	st	-	-		
5063			st	/	-		-	-	st	st	-	-		
5064			+	+	+		+(26,20)	+(24,90)	+	+	+	+		
5065			+	+	+		+(20,96)	+(19,93)	+	+	+	+		
5066			st	/	-		-	-	st	st	-	-		
5067			+	+	+		+(22,2)	+(21,88)	+	+	+	+		
5068			+	+	+		+(26,25)	+(25,21)	+	+	+	+		
5069			+	+	+		+(25,26)	+(25,13)	+	+	+	+		
4574		8,4	+	+	+	5/5	+(25,17)	+(24,49)	+	+	+	+	5/5	5/5
4575			+	+	+		+(23,31)	+(23,06)	+	+	+	+		
4576			+	+	+		+(28,53)	+(28,02)	+	+	+	+		
4577			+	+	+		+(23,8)	+(23,04)	+	+	+	+		
4578			+	+	+		+(23,63)	+(24,84)	+	+	+	+		

Process water
Strain: *Cronobacter turicensis* Ad 1445

Sample N°	Product	Inoculation level	Reference method : ISO 22964				Alternative method : SureTect *Cronobacter* species 18 h at 37°C ± 1°C							
			CCI	Confirmatory tests	Final result	Number positive samples/ Total	PCR result (Cq value)		Confirmatory tests		Final result Piko Real	Final result 7500 Fast	Number positive samples/ Total PikoReal	Number positive samples/ Total 7500 Fast
							Piko Real	7500 Fast	CCI (direct streaking)	CCI (streaking after subculture in CSB)				
4306	Process water(Rinse water during Milk powder production) Aerobic mesophilic flora: 6 CFU/ml	0 CFU/ml	st	/	-	0/5	-	-	st	st	-	-	0/5	0/5
4307			st	/	-		-	-	st	st	-	-		
4308			st	/	-		-	-	st	st	-	-		
4309			st	/	-		-	-	st	st	-	-		
4310			st	/	-		-	-	st	st	-	-		
4311		0,7 CFU/ml	+	+	+	8/20	+(21,73)	+(21,35)	+	+	+	+	9/20	9/20
4312			st	/	-		-	-	st	st	-	-		
4313			st	/	-		-	-	st	st	-	-		
4314			st	/	-		-	-	st	st	-	-		
4315			st	/	-		-	-	st	st	-	-		
4316			+	+	+		+(22,29)	+(19,41)	+	+	+	+		
4317			+	+	+		+(21,46)	+(20,00)	+	+	+	+		
4318			+	+	+		-	-	st	st	-	-		
4319			+	+	+		+(21,15)	+(19,53)	+	+	+	+		
4320			+	+	+		-	-	st	st	-	-		
4321			st	/	-		-	-	st	st	-	-		
4322			st	/	-		+(21,85)	+(20,31)	+	+	+	+		
4323			st	/	-		+(21,92)	+(20,15)	+	+	+	+		
4324			st	/	-		-	-	st	st	-	-		
4325			+	+	+		+(22,17)	+(20,15)	+	+	+	+		
4326			st	/	-		-	-	st	st	-	-		
4327			+	+	+		+(21,56)	+(20,15)	+	+	+	+		
4328			st	/	-		+(21,42)	+(20,05)	+	+	+	+		
4329			+	+	+		-	-	st	st	-	-		
4330			st	/	-		-	-	st	st	-	-		
4331		1,9 CfU/ml	+	+	+	4/5	+(20,68)	+(20,59)	+	+	+	+	3/5	3/5
4332			st	/	-		+(20,59)	+(20,17)	+	+	+	+		
4333			+	+	+		-	-	st	st	-	-		
4334			+	+	+		-	-	st	st	-	-		
4335			+	+	+		+(20,90)	+(20,28)	+	+	+	+		

Infant formula with probiotics (300 g sample size)
Strain: *Cronobacter sakazakii* Ad1446

Sample N°	Product	Inoculation level/sample	Reference method : ISO 22964				Alternative method : SureTect *Cronobacter* species BPW + Vancomycin (6 mg/L)-20 h at 37°C ± 1°C										
			CCI	Confirmatory tests	Final result	Number positive samples/ Total	PCR result (Cq value)			Confirmatory tests		Final result Piko Real	Final result 7500 Fast	Final result QS5	Number positive samples/ Total PikoReal	Number positive samples/ Total 7500 Fast	Number positive samples/ Total QS5
							Piko Real	7500 Fast	QS5	CCI (direct streaking)	CCI (streaking after subculture in CSB)						
3583	Infant formula with probiotics (Anaerobic lactic flora 9,2 10^5 CFU/g)	0	st		-	0/5	-	-	-	st	st	-	-	-	0/5	0/5	0/5
3584			st		-		-	-	-	st	st	-	-	-			
3585			st		-		!/-	-	-	st	st	-	-	-			
3586			st		-		-	-	-	st	st	-	-	-			
3587			st		-		-	-	-	st	st	-	-	-			
3950		1,5	st		-	13/20	-	-	-	+p	+p	-	-	-	12/20	12/20	12/20
3951			+p	+	+		+(31,88)	+(32,07)	+(33,26)	+p	+p	+	+	+			
3952			+p	+	+		+(36,18)	+(36,93)	+(38,04)	+p	+p	+	+	+			
3953			st		-		+(34,05)	+(35,87)	+(36,80)	+p	+p	+	+	+			
3954			st		-		+(35,53)	+(35,85)	+(36,18)	+p	+p	+	+	+			
4009			+p	+	+		-	-	-	-	+M	-	-	-			
4010			+p	+	+		+(37,17)	+(35,48)	+(37,69)	+p	+p	+	+	+			
4011			st		-		-	-/-	+(39,72)	st	+p	-	-	+			
4012			+p	+	+		-	-	-	st	+p	-	-	-			
4013			+p	+	+		-	-	-	st	+p	-	-	-			
4014			+p	+	+		-	+(37,64)	+(39,16)	+p	+p	-	+	+			
4015			st		-		+(34,79)	+(35,25)	+(36,50)	+p	+p	+	+	+			
4016			+p	+	+		+(35,70)	+(36,74)	+(37,76)	+p	+p	+	+	+			
4017			+p	+	+		-	-	-	st	st	-	-	-			
4018			+p	+	+		+(34,29)	+(34,88)	+(37,77)	+p	+p	+	+	+			
4019			+p	+	+		+(38,22)	+(38,82)/+ (38,67)	-	+p	+p	+	+	-			
4020			+p	+	+		+(31,65)	+(32,05)	+(33,79)	+p	+p	+	+	+			
4021			st		-		+(30,79)	+(31,31)	+(33,30)	+p	+p	+	+	+			
4022			st		-		-	-	-	st	st	-	-	-			
4023			+p	+	+		+(38,24)	-	-	+p	+p	+	-	-			
3608		3,2	+p	+	+	5/5	+(35,04)	+(34,49)	+(33,50)	+p	+p	+	+	+	3/5	3/5	3/5
3609			+p	+	+		-/-/-	+(39,43)/- /-	+(40,96)	+(6)	+p	-	+	+			
3610			+p	+	+		-	-	-	st	st	-	-	-			
3611			+p	+	+		+(36,25)	+(34,69)	+(38,49)	+1/2	+p	+	+	+			
3612			+p	+	+		-	-	-	st	st	-	-	-			

APPENDIX E - Relative level of detection - Extension study (2021)

Matrix: Infant cereals with probiotics **Enumeration of probiotics: 5,4.10^6 UFC/g**

Strain: *Cronobacter dublinensis* GVV828

Sample N°	Inoculation level / sample	ISO 22964 (■)				SureTect *Cronobacter spp* BPW + Vancomycin (6mg/L)-20h at 34-38°C						
		CCI	Confirmation tests	**Final result**	Number positive samples / Total	7500 Fast PCR result (Cq value)	QS5 PCR result (Cq value)	Confirmation tests	**Final result 7500 Fast**	**Final result QS5**	Number positive samples / Total 7500 FAST	Number positive samples / Total QS5
2045429	0	∅	/	**A**	0/5	-	-	/	**A**	**A**	0/5	0/5
2045430		∅	/	**A**		-	-	/	**A**	**A**		
2045431		∅	/	**A**		-	-	/	**A**	**A**		
2045432		∅	/	**A**		-	-	/	**A**	**A**		
2045433		∅	/	**A**		-	-	/	**A**	**A**		
2045409	2,0	AM	+	**P**	13/20	+ (21,46)	+ (19,99)	+	**P**	**P**	12/20	12/20
2045410		∅	/	**A**		-	-	/	**A**	**A**		
2045411		AM	+	**P**		+ (22,30)	+ (20,44)	+	**P**	**P**		
2045412		AM	+	**P**		+ (22,05)	+ (20,00)	+	**P**	**P**		
2045413		AM	+	**P**		+ (18,99)	+ (17,38)	+	**P**	**P**		
2045414		AM	+	**P**		-	-	/	**A**	**A**		
2045415		AM	+	**P**		+ (22,13)	+ (20,74)	+	**P**	**P**		
2045416		∅	/	**A**		+ (20,52)	+ (18,41)	+	**P**	**P**		
2045417		AM	+	**P**		-	-	/	**A**	**A**		
2045418		AM	+	**P**		+ (20,65)	+ (19,89)	+	**P**	**P**		
2045419		∅	/	**A**		+ (22,15)	+ (20,81)	+	**P**	**P**		
2045420		∅	/	**A**		-	-	/	**A**	**A**		
2045421		AM	+	**P**		+ (21,23)	+ (18,98)	/	**P**	**P**		
2045422		AM	+	**P**		-	-	/	**A**	**A**		
2045423		∅	/	**A**		+ (28,03)	+ (26,62)	+	**P**	**P**		
2045424		AM	+	**P**		+ (21,23)	+ (19,93)	+	**P**	**P**		
2045425		∅	/	**A**		+ (19,38)	+ (18,11)	+	**P**	**P**		
2045426		AM	+	**P**		-	-	/	**A**	**A**		
2045427		AM	+	**P**		-	-	/	**A**	**A**		
2045428		∅	/	**A**		-	-	/	**A**	**A**		
2045434	6,0	AM	+	**P**	5/5	+ (19,20)	+ (18,33)	+	**P**	**P**	5/5	5/5
2045435		AM	+	**P**		+ (20,37)	+ (19,61)	+	**P**	**P**		
2045436		AM	+	**P**		+ (21,03)	+ (19,82)	+	**P**	**P**		
2045437		AM	+	**P**		+ (20,24)	+ (19,23)	+	**P**	**P**		
2045438		AM	+	**P**		+ (20,82)	+ (19,51)	+	**P**	**P**		

Appendix F - Inclusivity / Exclusivity: raw data

INCLUSIVITY								
n°	Genus	Species	N°	Origin	Inoculation level CFU/225ml	BPW + vancomycin (6 mg/L) for 18 h at 37°C		
						SureTect *Cronobacter* species PCR result (Cq)		CCI (direct streaking)
						PikoReal	7500 Fast	
1	*Cronobacter*	*dublinensis*	DSM18705	Dairy Product	34	+(20.82)	+(18.35)	+
2	*Cronobacter*	*malonaticus*	DSM18702	Dairy Product	11	+(21.28)	+(19.51)	+
3	*Cronobacter*	*malonaticus*	Ad1708	Dairy Product	53	+(21.41)	+(19.53)	+
4	*Cronobacter*	*muytjensii*	CIP103581	/	31	+(20.92)	+(18.24)	+
5	*Cronobacter*	*sakazakii*	Ad939	Infant formula	42	+(21.13)	+(19.12)	+
6	*Cronobacter*	*sakazakii*	Ad940	Infant formula	120	+(21.09)	+(19.76)	+
7	*Cronobacter*	*sakazakii*	Ad941	Infant formula	67	+(20.73)	+(19.24)	+
8	*Cronobacter*	*sakazakii*	Ad942	Infant formula	89	+(20.14)	+(19.09)	+
9	*Cronobacter*	*sakazakii*	Ad943	Infant formula	28	+(21.07)	+(19.58)	+
10	*Cronobacter*	*sakazakii*	Ad944	Infant formula	31	+(21.29)	+(19.05)	+
11	*Cronobacter*	*sakazakii*	Ad945	Infant formula	36	+(21.52)	+(19.18)	+
12	*Cronobacter*	*sakazakii*	Ad946	Infant formula	50	+21.52)	+(19.42)	+
13	*Cronobacter*	*sakazakii*	Ad947	Infant formula	46	+(21.77)	+(19.55)	+
14	*Cronobacter*	*sakazakii*	Ad948	Infant formula	47	+(20.59)	+(19.14)	+
15	*Cronobacter*	*sakazakii*	Ad949	Infant formula	71	+(21.66)	+(19.49)	+
16	*Cronobacter*	*sakazakii*	Ad950	Infant formula	49	+(21.25)	+(19.17)	+
17	*Cronobacter*	*sakazakii*	Ad951	Infant formula	41	+(21.74)	+(18.57)	+
18	*Cronobacter*	*sakazakii*	Ad952	Infant formula	37	+(21.30)	+(19.49)	+
19	*Cronobacter*	*sakazakii*	Ad953	Infant formula	29	+(21.63)	+(20.24)	+
20	*Cronobacter*	*sakazakii*	Ad963	Infant formula	18	+(21.85)	+(19.68)	+
21	*Cronobacter*	*sakazakii*	Ad704	Infant formula	26	+(21.28)	+(19.11)	+
22	*Cronobacter*	*sakazakii*	Ad831	Infant formula	12	+(21.40)	+(19.44)	+
23	*Cronobacter*	*sakazakii*	Ad829	Infant formula	23	+(21.59)	+(19.26)	+
24	*Cronobacter*	*sakazakii*	Ad916	Infant formula	17	+(20.73)	+(19.50)	+
25	*Cronobacter*	*sakazakii*	Ad893	Infant formula	26	+(20.35)	+(19.48)	+
26	*Cronobacter*	*sakazakii*	Ad894	Infant formula	14	+(21.20)	+(19.84)	+
27	*Cronobacter*	*sakazakii*	Ad895	Infant formula	28	+(20.73)	+(19.32)	+ (blue green)
28	*Cronobacter*	*sakazakii*	Ad896	Infant formula	28	+(20.74)	+(19.59)	+
29	*Cronobacter*	*sakazakii*	Ad897	Infant formula	2	+(20.88)	+(19.54)	+
30	*Cronobacter*	*sakazakii*	Ad898	Infant formula	17	+(21.11)	+(19.75)	+

INCLUSIVITY								
n°	Genus	Species	N°	Origin	Inoculation level CFU/225ml	BPW + vancomycin (6 mg/L) for 18 h at 37°C		
						SureTect *Cronobacter* species PCR result (Cq)		CCI (direct streaking)
						PikoReal	7500 Fast	
31	*Cronobacter*	*dublinensis lactaridi*	DSMZ18707 T	Dairy Product	20	+(21.0)	+(19.40)	+
32	*Cronobacter*	*dublinensis lausannensis*	DSMZ 18706 T	Dairy Product	16	+(20.65)	+(19.28)	+
33	*Cronobacter*	*sakazakii*	Ad1418	Infant formula	18	+(20.65)	+(19.61)	+
34	*Cronobacter*	*sakazakii*	Ad1419	Infant formula	21	+(20.64)	+(19.57)	+
35	*Cronobacter*	*sakazakii*	Ad1420	Infant formula	27	+(20.96)	+(19.95)	+
36	*Cronobacter*	*sakazakii*	Ad1421	Infant formula	10	+(20.98)	+(20.01)	+
37	*Cronobacter*	*sakazakii*	Ad1424	Infant formula	12	+(21.16)	+(19.62)	+
38	*Cronobacter*	*sakazakii*	Ad1425	Infant formula	13	+(20.33)	+(19.39)	+
39	*Cronobacter*	*sakazakii*	Ad1426	Infant formula	28	+(22.31)	+(19.72)	+
40	*Cronobacter*	*sakazakii*	Ad1427	Infant formula	32	+(20.66)	+(19.70)	+
41	*Cronobacter*	*sakazakii*	Ad1428	Infant formula	24	+(19.67)	+(20.36)	+
42	*Cronobacter*	*sakazakii*	Ad1429	Infant formula	27	+(22.66)	+(22.34)	+
43	*Cronobacter*	*sakazakii*	Ad1430	Infant formula	24	+(20.10)	+(20.19)	+
44	*Cronobacter*	*sakazakii*	Ad1431	Infant formula	31	+(19.88)	+(20.53)	+
45	*Cronobacter*	*sakazakii*	Ad1432	Infant formula	24	+(20.10)	+(20.01)	+
46	*Cronobacter*	*sakazakii*	Ad1433	Infant formula	31	+(20.12)	+(19.96)	+
47	*Cronobacter*	*sakazakii*	Ad1434	Infant formula	30	+(20.79)	+(19.56)	+
48	*Cronobacter*	*sakazakii*	Ad1435	Infant formula	30	+(20.74)	+(20.29)	+
49	*Cronobacter*	*turicencis*	Ad 1445	Infant formula	17	+(20.35)	+(21.51)	+
50	*Cronobacter*	*turicencis*	DSMZ 18703	/	15	+(20.32)	+(21.02)	+
51	*Cronobacter*	*malonaticus*	E752	Baby food	17	+(21.31)	+(19.41)	+
52	*Cronobacter*	*turicensis*	E681	Food	15	+(27.46)	+(20.21)	+
53	*Cronobacter*	*muytjensii*	E769	Milk powder	9	+(26.95)	+(20.05)	+
54	*Cronobacter*	*dublinensis* subsp *dublinensis*	LMG 23823T	Environment	20	+(21.40)	+(20.43)	+
55	*Cronobacter*	*dublinensis* subsp *lausanensis*	E798	/	22	+(22.64)	+(25.01)	+
56	*Cronobacter*	*universalis*	NCTC 9529T	water	31	+(21.12)	+(20.31)	+
57	*Cronobacter*	*condimenti*	LMG 26250T	Spiced meat	15	+(21.34)	+(19.17)	+

EXCLUSIVITY							
N°	Genus	Species	Reference	Origin	Inoculation level CFU/225ml	BPW for 24 h at 37°C	
						SureTect *Cronobacter* species PCR result (Cq)	
						PikoReal	7500 Fast
1	*Citrobacter*	*braakii*	Ad833	Beef	2.40E+05	-	-
2	*Citrobacter*	*diversus*	Ad173	Dairy product	2.00E+05	-	-
3	*Citrobacter*	*fameri*	Ad116	Environmental sample	3.00E+05	-	-
4	*Citrobacter*	*freundi*	39	Environmental sample	2.60E+05	-	-
5	*Citrobacter*	*koseri*	CIP105177	/	3.30E+05	-	-
6	*Enterobacter*	*aerogenes*	Ad889	Meat flour	2.30E+05	-	-
7	*Enterobacter*	*agglomerans*	11	Dairy product	2.20E+05	-	-
8	*Enterobacter*	*agglomerans*	136	Dairy product	1.70E+05	-	-
9	*Enterobacter*	*amnigenus*	52	Vegetables	7.20E+05	-	-
10	*Enterobacter*	*amnigenus*	129	Raw milk	1.70E+05	-	-
11	*Enterobacter*	*amnigenus*	A00C068	Poultry	3.40E+05	-	-
12	*Enterobacter*	*cloacae*	51	Vegetables	3.60E+05	-	-
13	*Enterobacter*	*cloacae*	10	Dairy product	1.60E+05	-	-
14	*Enterobacter*	*fergusonii*	2876	Environmental sample	3.10E+05	-	-
15	*Enterobacter*	*gergoviae*	CIP76.1	/	2.80E+05	-	-
16	*Franconibacter*	*helveticus*	DSM 18396 T	fruit powder	1.70E+05	-	-
17	*Enterobacter*	*hormaechei*	Ad990	Butter	2.60E+05	-	-
18	*Enterobacter*	*intermedius*	60	Vegetables	1.10E+05	-	-
19	*Enterobacter*	*kobei*	Ad706	Milk powder	4.00E+05	-	-
20	*Escherichia*	*coli*	16	Dairy product	4.10E+05	-	-
21	*Escherichia*	*hermanii*	Ad462	Dairy product	1.90E+05	-	-
22	*Hafnia*	*alvei*	Ad245	Dairy product	3.70E+05	-	-
23	*Klebsiella*	*pneumoniae*	122	Dairy product	2.00E+05	-	-
24	*Leclercia*	*adecarboxylata*	Ad707	Milk powder	6.60E+04	-	-
25	*Salmonella*	*arizonae* (51:z4,z23)	CIP 5523	/	3.00E+05	-	-
26	*Salmonella*	*diarizonae* SIIIb 65 :c :z	Ad 1298	Dairy environmental sample	3.60E+05	-	-
27	*Salmonella*	Typhimurium	Ad1333	Dairy product	5.30E+05	-	-
28	*Serratia*	*ficaria*	113	Vegetables	3.30E+05	-	-
29	*Serratia*	*marcescens*	Ad455	Raw milk	3.60E+05	-	-
30	*Yersinia*	*intermedia*	Ad133	Dairy product	3.20E+05	-	-
31	*Escherichia*	*coli O103*	Ad 1862	Dairy product	2.80E+05	-	-

Appendix G – Interlaboratory study raw results

Microbact galleries were provided to run the characteristic colonies confirmation. Some Labs encountered difficulties in the galleries interpretation. Despite the identifications not fitting with the expected results, their results were taken into account based on the recovery of characteristic colonies. Note that the characteristic colonies were identified correctly as *Cronobacter* spp. by the labs familiar with the Microbact gallery.

Laboratory **A**

Lactic flora: 5.6 x10^4/g

N° Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
A2	-	/	/	/	-	/	33.33	-	-	-	/	-	**NA**
A6	-	/	/	/	-	/	33.41	-	-	-	/	-	**NA**
A8	-	/	/	/	-	/	34.36	-	-	-	/	-	**NA**
A11	-	/	/	/	-	/	33.31	-	-	-	/	-	**NA**
A15	-	/	/	/	-	/	34.53	-	-	-	/	-	**NA**
A19	-	/	/	/	-	/	33.98	-	-	-	/	-	**NA**
A22	-	/	/	/	-	/	32.57	-	-	-	/	-	**NA**
A23	-	/	/	/	-	/	34.02	-	-	-	/	-	**NA**
A3	+	+	-	/	+	24.4	34.4	+	+	+	*Hafnia alvei*	+	**PA**
A7	+	+	-	/	+	23.6	33.84	+	+	+	*Serratia maltophilia*	+	**PA**
A9	+	+	-	/	+	22.77	35.39	+	+	+	*Hafnia alvei/Serratia maltophilia*	+	**PA**
A13	-	/	/	/	-	/	33.38	-	-	-	/	-	**NA**
A16	+	+	-	/	+	22.38	35.03	+	+	+	*Hafnia alvei/Serratia maltophilia*	+	**PA**
A18	+	+	-	/	+	22.89	33.8	+	+	+	*E.coli*	+	**PA**
A21	+	+	-	/	+	22.1	35.29	+	+	+	*Hafnia alvei*	+	**PA**
A24	+	+	-	/	+	22.32	37.24	+	+	+	*E.coli*	+	**PA**
A1	+	+	-	/	+	22.41	34.5	+	+	+	*Hafnia alvei*	+	**PA**
A4	+	+	-	/	+	23.48	34.67	+	+	+	*Hafnia alvei*	+	**PA**
A5	+	+	-	/	+	23.54	34.12	+	+	+	*Serratia maltophilia*	+	**PA**
A10	+	+	-	/	+	23.16	34.34	+	+	+	*Hafnia alvei/Serratia maltophilia*	+	**PA**
A12	+	+	-	/	+	23.59	34.03	+	+	+	*Hafnia alvei/Serratia maltophilia*	+	**PA**
A14	+	+	-	/	+	24.03	34.91	+	+	+	*Hafnia alvei*	+	**PA**
A17	+	+	-	/	+	23.22	35.31	+	+	+	*E.coli*	+	**PA**
A20	+	+	-	/	+	24.24	34.18	+	+	+	*Hafnia alvei*	+	**PA**

Laboratory **B**

Lactic flora: 8.3 x10^4/g

N° Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
B2	-	/	/	/	-	-	33.22	-	-	-	/	-	**NA**
B6	-	/	/	/	-	-	34.22	-	-	-	/	-	**NA**
B8	-	/	/	/	-	-	33.77	-	-	-	/	-	**NA**
B11	-	/	/	/	-	-	32.87	-	-	-	/	-	**NA**
B15	-	/	/	/	-	-	32.88	-	-	-	/	-	**NA**
B19	-	/	/	/	-	-	32.65	-	-	-	/	-	**NA**
B22	-	/	/	/	-	-	34,00	-	-	-	/	-	**NA**
B23	-	/	/	/	-	-	33.51	-	-	-	/	-	**NA**
B3	+	+	-	*Cronobacter sakazakii*	+	23.44	35.05	+	+	+	*Cronobacter sakazakii*	+	**PA**
B7	-	/	/	/	-	-	33.99	-	-	-	/	-	**NA**
B9	+	+	-	*Cronobacter sakazakii*	+	21.97	35.1	+	+	+	*Cronobacter sakazakii*	+	**PA**
B13	-	/	/	/	-	-	34.21	-	-	-	/	-	**NA**
B16	+	+	-	*Cronobacter sakazakii*	+	22.2	34.62	+	+	+	*Cronobacter sakazakii*	+	**PA**
B18	+	+	-	*Cronobacter sakazakii*	+	22.16	34.83	+	+	+	*Cronobacter sakazakii*	+	**PA**
B21	-	/	/	/	-	-	34.14	-	-	-	/	-	**NA**
B24	+	+	-	*Cronobacter sakazakii*	+	21.63	38.58	+	+	+	*Cronobacter sakazakii*	+	**PA**
B1	+	+	-	*Cronobacter sakazakii*	+	21.57	33.06	+	+	+	*Cronobacter sakazakii*	+	**PA**
B4	+	+	-	*Cronobacter sakazakii*	+	21.37	32.56	+	+	+	*Cronobacter sakazakii*	+	**PA**
B5	+	+	-	*Cronobacter sakazakii*	+	21.88	33.23	+	+	+	*Cronobacter sakazakii*	+	**PA**
B10	+	+	-	*Cronobacter sakazakii*	+	21.38	32.71	+	+	+	*Cronobacter sakazakii*	+	**PA**
B12	+	+	-	*Cronobacter sakazakii*	+	21.5	32.99	+	+	+	*Cronobacter sakazakii*	+	**PA**
B14	+	+	-	*Cronobacter sakazakii*	+	21.66	33.3	+	+	+	*Cronobacter sakazakii*	+	**PA**
B17	+	+	-	*Cronobacter sakazakii*	+	21.09	34.24	+	+	+	*Cronobacter sakazakii*	+	**PA**
B20	+	+	-	*Cronobacter sakazakii*	+	20.53	34.07	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **C**

Lactic flora: 8.4 x10^4/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: Sure Tect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/CCI Agar	Biochemical galleries		
C2	-	/	/	/	-	/	34.62	-	-	-	/	-	**NA**
C6	-	/	/	/	-	/	33.5	-	-	-	/	-	**NA**
C8	-	/	/	/	-	/	34.66	-	-	-	/	-	**NA**
C11	-	/	/	/	-	/	34.46	-	-	-	/	-	**NA**
C15	-	/	/	/	-	/	34.65	-	-	-	/	-	**NA**
C19	-	/	/	/	-	/	33.26	-	-	-	/	-	**NA**
C22	-	/	/	/	-	/	34.19	-	-	-	/	-	**NA**
C23	-	/	/	/	-	/	34.2	-	-	-	/	-	**NA**
C3	-	/	/	/	-	/	33.87	-	-	-	/	-	**NA**
C7	-	/	/	/	-	/	34.18	-	-	-	/	-	**NA**
C9	-	/	/	/	-	/	34.32	-	-	-	/	-	**NA**
C13	-	/	/	/	-	/	32.99	-	-	-	/	-	**NA**
C16	+	+	-	*Hafnia alvei*	+	20.47	37.29	+	+	+	*Hafnia alvei*	+	**PA**
C18	+	+	-	*Hafnia alvei*	+	21.33	34.87	+	+	+	*Hafnia alvei*	+	**PA**
C21	+	+	-	*Hafnia alvei*	+	21.78	34.73	+	+	+	*Hafnia alvei*	+	**PA**
C24	+	+	-	*Hafnia alvei*	+	20.84	34.76	+	+	+	*Hafnia alvei*	+	**PA**
C1	+	+	-	*Hafnia alvei*	+	21.03	35.04	+	+	+	*Hafnia alvei*	+	**PA**
C4	+	+	-	*Hafnia alvei*	+	21.53	34.12	+	+	+	*Hafnia alvei*	+	**PA**
C5	+	+	-	*Hafnia alvei*	+	20.71	33,00	+	+	+	*Hafnia alvei*	+	**PA**
C10	+	+	-	*Hafnia alvei*	+	21.98	34.22	+	+	+	*Hafnia alvei*	+	**PA**
C12	+	+	-	*Hafnia alvei*	+	22.34	34.24	+	+	+	*Hafnia alvei*	+	**PA**
C14	+	+	-	*Hafnia alvei*	+	21.59	33.1	+	+	+	*Hafnia alvei*	+	**PA**
C17	+	+	-	*Hafnia alvei*	+	21.31	34.13	+	+	+	*Hafnia alvei*	+	**PA**
C20	+	+	-	*Hafnia alvei*	+	21.78	34.35	+	+	+	*Hafnia alvei*	+	**PA**

Laboratory **D**

Lactic flora: 8.3 x10^4 /g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
D2	-	/	/	/	-	/	35.16	-	-	-	/	-	**NA**
D6	-	/	/	/	-	/	34.48	-	-	-	/	-	**NA**
D8	-	/	/	/	-	35.33	/	+	-	-/-*	/	-	**PPNA**
D11	-	/	/	/	-	/	35.17	-	+	+/-*	*Cronobacter sakazakii*	-	**NA**
D15	-	/	/	/	-	/	34.31	-	+	+/-*	*Cronobacter sakazakii*	-	**NA**
D19	-	/	/	/	-	/	34.32	-	-	-	/	-	**NA**
D22	+	+	-	*Cronobacter sakazakii*	+	/	33.51	-	-	-/-*	/	-	**ND**
D23	+	+	-	*Cronobacter sakazakii*	+	39.36	/	+	-	-/-*	/	-	**PPND**
D3	-	/	/	/	-	/	33.97	-	-	-	/	-	**NA**
D7	+	+	-	*Cronobacter sakazakii*	+	18.32	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D9	+	+	-	*E.coli*	+	19.87	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D13	+	+	-	*Klebsiella ozeanae*	+	22.57	/	+	+	+	*E.coli*	+	**PA**
D16	+	+	-	*Serratia liquefaciens*	+	20.36	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D18	-	/	/	/	-	/	34.64	-	-	-	/	-	**NA**
D21	+	+	-	*Cronobacter sakazakii*	+	20.39	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D24	+	+	-	*Cronobacter sakazakii*	+	35.09	/	+	-	-/-*	/	-	**PPND**
D1	+	+	-	*Enterobacter aerogenes*	+	20.32	/	+	+	+	*E.coli*	+	**PA**
D4	+	+	-	*Cronobacter sakazakii*	+	20.37	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D5	+	+	-	*E.coli*	+	21.01	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D10	+	+	-	*Serratia liquefaciens*	+	20.21	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D12	+	+	-	*E.coli*	+	20.31	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D14	+	+	-	*Cronobacter sakazakii*	+	19.82	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D17	+	+	-	*Cronobacter sakazakii*	+	20.41	/	+	+	+	*Cronobacter sakazakii*	+	**PA**
D20	+	+	-	*Cronobacter sakazakii*	+	20.4	/	+	+	+	*Klebsiella ozeanae*	+	**PA**

*: second streaking

Laboratory **E**

Lactic flora: 5.3 x10⁴/g

N° Sample	Reference method: ISO/TS 22964					Alternative method: Sure Tect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
E2	-	/	/	/	-	/	34.7	-	-	-	/	-	**NA**
E6	-	/	/	/	-	/	33.71	-	-	-	/	-	**NA**
E8	-	/	/	/	-	/	34.8	-	-	-	/	-	**NA**
E11	-	/	/	/	-	/	34.5	-	-	-	/	-	**NA**
E15	-	/	/	/	-	/	34.06	-	-	-	/	-	**NA**
E19	-	/	/	/	-	38.32	34.11	+	-	-	/	-	**PPNA**
E22	-	/	/	/	-	/	30.93	-	-	-	/	-	**NA**
E23	-	/	/	/	-	/	31.91	-	-	-	/	-	**NA**
E3	+	+	-	*Cronobacter sakazakii*	+	22.3	33.44	+	+	+	*Cronobacter sakazakii*	+	**PA**
E7	+	+	-	*Cronobacter sakazakii*	+	22.23	33.86	+	+	+	*Cronobacter sakazakii*	+	**PA**
E9	+	+	-	*Cronobacter sakazakii*	+	21.53	34.26	+	+	+	*Cronobacter sakazakii*	+	**PA**
E13	-	/	/	/	-	/	33.39	-	-	-	/	-	**NA**
E16	+	+	-	*Cronobacter sakazakii*	+	21.36	34.76	+	+	+	*Cronobacter sakazakii*	+	**PA**
E18	-	/	/	/	-	/	32.6	-	-	-	/	-	**NA**
E21	-	/	/	/	-	/	34.02	-	-	-	/	-	**NA**
E24	+	+	-	*Cronobacter sakazakii*	+	21.63	33.68	+	+	+	*Cronobacter sakazakii*	+	**PA**
E1	+	+	-	*Cronobacter sakazakii*	+	19.83	34.22	+	+	+	*Cronobacter sakazakii*	+	**PA**
E4	+	+	-	*Cronobacter sakazakii*	+	20.1	34.46	+	+	+	*Cronobacter sakazakii*	+	**PA**
E5	+	+	-	*Cronobacter sakazakii*	+	20.17	34.38	+	+	+	*Cronobacter sakazakii*	+	**PA**
E10	+	+	-	*Cronobacter sakazakii*	+	20.89	31.24	+	+	+	*Cronobacter sakazakii*	+	**PA**
E12	+	+	-	*Cronobacter sakazakii*	+	22.97	33.53	+	+	+	*Cronobacter sakazakii*	+	**PA**
E14	+	+	-	*Cronobacter sakazakii*	+	20.83	34.23	+	+	+	*Cronobacter sakazakii*	+	**PA**
E17	+	+	-	*Cronobacter sakazakii*	+	20.34	35.3	+	+	+	*Cronobacter sakazakii*	+	**PA**
E20	+	+	-	*Cronobacter sakazakii*	+	21.34	34.62	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **F**

Lactic flora flora: 9.2 x10^4/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
F2	-	/	/	/	-	/	33.25	-	-	-	/	-	**NA**
F6	-	/	/	/	-	/	33.11	-	-	-	/	-	**NA**
F8	-	/	/	/	-	/	34.02	-	-	-	/	-	**NA**
F11	-	/	/	/	-	/	33.36	-	-	-	/	-	**NA**
F15	-	/	/	/	-	/	33.33	-	-	-	/	-	**NA**
F19	-	/	/	/	-	/	33.48	-	-	-	/	-	**NA**
F22	-	/	/	/	-	/	33.06	-	-	-	/	-	**NA**
F23	-	/	/	/	-	/	33.23	-	-	-	/	-	**NA**
F3	+	+	-	*Cronobacter sakazakii*	+	24.16	35.35	+	+	+	*Cronobacter sakazakii*	+	**PA**
F7	-	/	/	/	-	/	33.95	-	-	-	/	-	**NA**
F9	+	+	-	*Cronobacter sakazakii*	+	22.17	35.32	+	+	+	*Cronobacter sakazakii*	+	**PA**
F13	+	+	-	*Cronobacter sakazakii*	+	24.16	34.11	+	+	+	*Cronobacter sakazakii*	+	**PA**
F16	-	/	/	/	-	/	33.58	-	-	-	/	-	**NA**
F18	+	+	-	*Cronobacter sakazakii*	+	25.35	33.84	+	+	+	*Cronobacter sakazakii*	+	**PA**
F21	-	/	/	/	-	/	32.98	-	-	-	/	-	**NA**
F24	+	+	-	*Cronobacter sakazakii*	+	24.14	34.94	+	+	+	*Cronobacter sakazakii*	+	**PA**
F1	+	+	-	*Cronobacter sakazakii*	+	20.51	34.9	+	+	+	*Cronobacter sakazakii*	+	**PA**
F4	+	+	-	*Cronobacter sakazakii*	+	20.63	33.63	+	+	+	*Cronobacter sakazakii*	+	**PA**
F5	+	+	-	*Cronobacter sakazakii*	+	21.58	34.44	+	+	+	*Cronobacter sakazakii*	+	**PA**
F10	+	+	-	*Cronobacter sakazakii*	+	21.52	34.73	+	+	+	*Cronobacter sakazakii*	+	**PA**
F12	+	+	-	*Cronobacter sakazakii*	+	22.48	34.04	+	+	+	*Cronobacter sakazakii*	+	**PA**
F14	+	+	-	*Cronobacter sakazakii*	+	21.04	34.15	+	+	+	*Cronobacter sakazakii*	+	**PA**
F17	+	+	-	*Cronobacter sakazakii*	+	22.86	36.79	+	+	+	*Cronobacter sakazakii*	+	**PA**
F20	+	+	-	*Cronobacter sakazakii*	+	21.8	34.9	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **G**

Lactic flora: 1.9 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: Sure Tect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
G2	-	/	/	/	-	/	33.7	-	-	-	/	-	**NA**
G6	-	/	/	/	-	/	34.41	-	-	-	/	-	**NA**
G8	-	/	/	/	-	/	33.51	-	-	-	/	-	**NA**
G11	-	/	/	/	-	/	33.29	-	-	-	/	-	**NA**
G15	+	+	-	*Cronobacter sakazakii*	+	/	33.33	-	-	-	/	-	**ND**
G19	-	/	/	/	-	/	33.54	-	-	-	/	-	**NA**
G22	-	/	/	/	-	/	34.11	-	-	-	/	-	**NA**
G23	-	/	/	/	-	/	34.49	-	-	-	/	-	**NA**
G3	-	/	/	/	-	/	35.08	-	-	-	/	-	**NA**
G7	+	+	-	*Cronobacter sakazakii*	+	21.73	34.38	+	+	-	+	+	**PA**
G9	+	+	-	*Cronobacter sakazakii*	+	21.68	35.01	+	+	-	+	+	**PA**
G13	-	/	/	/	-	/	32.85	-	-	-	/	-	**NA**
G16	-	/	/	/	-	19.6	34.28	+	+	+	*Cronobacter sakazakii*	+	**PD**
G18	+	+	-	*Cronobacter sakazakii*	+	20.02	33.87	+	+	+	*Cronobacter sakazakii*	+	**PA**
G21	-	/	/	/	-	/	34.63	-	-	-	/	-	**NA**
G24	+	+	-	*Cronobacter sakazakii*	+	23.49	34.62	+	+	+	*Cronobacter sakazakii*	+	**PA**
G1	+	+	-	+	+	21.09	34.56	+	+	-	+	+	**PA**
G4	+	+	-	*Cronobacter sakazakii*	+	21,00	34.29	+	+	-	+	+	**PA**
G5	+	+	-	*Cronobacter sakazakii*	+	20.56	35.01	+	+	+	*Cronobacter sakazakii*	+	**PA**
G10	+	+	-	*Cronobacter sakazakii*	+	21.03	34.09	+	+	+	*Cronobacter sakazakii*	+	**PA**
G12	+	+	-	*Cronobacter sakazakii*	+	19.09	33.98	+	+	+	*Cronobacter sakazakii*	+	**PA**
G14	+	+	-	*Cronobacter sakazakii*	+	20.72	34.28	+	+	+	*Cronobacter sakazakii*	+	**PA**
G17	+	+	-	*Cronobacter sakazakii*	+	20.67	35.18	+	+	+	*Cronobacter sakazakii*	+	**PA**
G20	+	+	-	*Cronobacter sakazakii*	+	19.42	33.66	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **H**

Lactic flora: 9.5 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
H2	-	/	/	/	-	/	33.8	-	-	-	/	-	**NA**
H6	-	/	/	/	-	/	33.8	-	-	-	/	-	**NA**
H8	-	/	/	/	-	/	33.6	-	-	-	/	-	**NA**
H11	-	/	/	/	-	/	34.05	-	-	-	/	-	**NA**
H15	-	/	/	/	-	/	33.49	-	-	-	/	-	**NA**
H19	-	/	/	/	-	/	34.27	-	-	-	/	-	**NA**
H22	-	/	/	/	-	/	34.13	-	-	-	/	-	**NA**
H23	-	/	/	/	-	/	34.06	-	-	-	/	-	**NA**
H3	+	+	-	*Cronobacter sakazakii*	+	21.11	31.96	+	+	+	*Cronobacter sakazakii*	+	**PA**
H7	+	+	-	*Cronobacter sakazakii*	+	21.48	33.12	+	+	+	*Cronobacter sakazakii*	+	**PA**
H9	+	+	-	*Cronobacter sakazakii*	+	21.76	33.83	+	+	+	*Cronobacter sakazakii*	+	**PA**
H13	-	/	/	/	-	/	33.47	-	-	-	/	-	**NA**
H16	-	/	/	/	-	/	33.98	-	-	-	/	-	**NA**
H18	+	+	-	*Cronobacter sakazakii*	+	20.8	33.9	+	+	+	*Cronobacter sakazakii*	+	**PA**
H21	-	/	/	/	-	/	34.33	-	-	-	/	-	**NA**
H24	-	/	/	/	-	/	33.18	-	-	-	/	-	**NA**
H1	+	+	-	*Cronobacter sakazakii*	+	19.94	34.35	+	+	+	*Cronobacter sakazakii*	+	**PA**
H4	+	+	-	*Cronobacter sakazakii*	+	20.49	31.96	+	+	+	*Cronobacter sakazakii*	+	**PA**
H5	+	+	-	*Cronobacter sakazakii*	+	21.51	33.09	+	+	+	*Cronobacter sakazakii*	+	**PA**
H10	+	+	-	*Cronobacter sakazakii*	+	20.79	33.5	+	+	+	*Cronobacter sakazakii*	+	**PA**
H12	+	+	-	*Cronobacter sakazakii*	+	20.45	32.62	+	+	+	*Cronobacter sakazakii*	+	**PA**
H14	+	+	-	*Cronobacter sakazakii*	+	20.6	33.46	+	+	+	*Cronobacter sakazakii*	+	**PA**
H17	+	+	-	*Cronobacter sakazakii*	+	20.23	34.8	+	+	+	*Cronobacter sakazakii*	+	**PA**
H20	+	+	-	*Cronobacter sakazakii*	+	20.21	32.71	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **I**

Lactic flora: 9.8 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
I2	-	/	/	/	-	/	35.9	-	-	-	/	-	NA
I6	-	/	/	/	-	/	36.14	-	-	-	/	-	NA
I8	-	/	/	/	-	/	33.38	-	-	-	/	-	NA
I11	-	/	/	/	-	/	34.32	-	-	-	/	-	NA
I15	-	/	/	/	-	/	35.31	-	-	-	/	-	NA
I19	-	/	/	/	-	/	34.96	-	-	-	/	-	NA
I22	-	/	/	/	-	/	35.12	-	-	-	/	-	NA
I23	-	/	/	/	-	/	35.09	-	-	-	/	-	NA
I3	-	/	/	/	-	/	36.87	-	-	-	/	-	NA
I7	-	/	/	/	-	/	35.1	-	-	-	/	-	NA
I9	-	/	/	/	-	/	33.71	-	-	-	/	-	NA
I13	-	/	/	/	-	/	35.75	-	-	-	/	-	NA
I16	+	+	-	*Klebsiella ozeanae*	+	21.08	34.18	+	+	+	*Serratia marcescens*	+	PA
I18	-	/	/	/	-	/	33.85	-	-	-	/	-	NA
I21	+	+	-	*Klebsiella ozeanae*	+	20.34	38.75	+	+	+	*C.lapagei*	+	PA
I24	+	+	-	*Serratia maltophilia*	+	20.56	39.29	+	+	+	*Serratia marcescens*	+	PA
I1	+	+	-	*Serratia maltophilia*	+	21.1	37.92	+	+	+	*Serratia maltophilia*	+	PA
I4	+	+	-	*Serratia maltophilia*	+	21.24	34.08	+	+	+	*Serratia maltophilia*	+	PA
I5	+	+	-	*Serratia maltophilia*	+	19.56	42.27	+	+	+	*Serratia maltophilia*	+	PA
I10	+	+	-	*Hafnia alvei*	+	20,00	39.08	+	+	+	*C.lapagei*	+	PA
I12	+	+	-	*Hafnia alvei*	+	22.72	48.05	+	+	+	*Hafnia alvei*	+	PA
I14	+	+	-	*C.lapagei*	+	22.05	33.22	+	+	+	*Serratia maltophilia*	+	PA
I17	+	+	-	*Serratia maltophilia*	+	22.16	33.88	+	+	+	*Serratia marcescens*	+	PA
I20	+	+	-	*Hafnia alvei*	+	20.52	39.01	+	+	+	*Cedecea spp*	+	PA

I13*: sample leaked

Laboratory **J**

Lactic flora: 5.1 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
J2	-	/	/	/	-	/	34.33	-	-	-	/	-	**NA**
J6	-	/	/	/	-	/	33.84	-	-	-	/	-	**NA**
J8	-	/	/	/	-	/	34.13	-	-	-	/	-	**NA**
J11	-	/	/	/	-	/	34.24	-	-	-	/	-	**NA**
J15	-	/	/	/	-	/	33.36	-	-	-	/	-	**NA**
J19	-	/	/	/	-	/	35.13	-	-	-	/	-	**NA**
J22	-	/	/	/	-	/	33.46	-	-	-	/	-	**NA**
J23	-	/	/	/	-	/	34.37	-	-	-	/	-	**NA**
J3	+	+	-	*Cronobacter sakazakii*	+	23.45	35.97	+	+	+	*Cronobacter sakazakii*	+	**PA**
J7	-	/	/	/	-	/	34.73	-	-	-	/	-	**NA**
J9	-	/	/	/	-	/	33.81	-	-	-	/	-	**NA**
J13	+	+	-	*Cronobacter sakazakii*	+	23.11	33.59	+	+	+	*Cronobacter sakazakii*	+	**PA**
J16	+	+	-	*Cronobacter sakazakii*	+	24.53	33.61	+	+	+	*Cronobacter sakazakii*	+	**PA**
J18	+	+	-	*Cronobacter sakazakii*	+	22.77	34.95	+	+	+	*Cronobacter sakazakii*	+	**PA**
J21	-	/	/	/	-	/	34.77	-	-	-	/	-	**NA**
J24	+	+	-	*Cronobacter sakazakii*	+	22.58	34.11	+	+	+	*Cronobacter sakazakii*	+	**PA**
J1	+	+	-	*Cronobacter sakazakii*	+	20.57	33.35	+	+	+	*Cronobacter sakazakii*	+	**PA**
J4	+	+	-	*Cronobacter sakazakii*	+	21.76	33.43	+	+	+	*Cronobacter sakazakii*	+	**PA**
J5	+	+	-	*Cronobacter sakazakii*	+	22.12	34.44	+	+	+	*Cronobacter sakazakii*	+	**PA**
J10	+	+	-	*Cronobacter sakazakii*	+	19.79	33.91	+	+	+	*Cronobacter sakazakii*	+	**PA**
J12	+	+	-	*Cronobacter sakazakii*	+	21.58	34.39	+	+	+	*Cronobacter sakazakii*	+	**PA**
J14	+	+	-	*Cronobacter sakazakii*	+	22.59	35.56	+	+	+	*Cronobacter sakazakii*	+	**PA**
J17	+	+	-	*Cronobacter sakazakii*	+	22.41	34.74	+	+	+	*Cronobacter sakazakii*	+	**PA**
J20	+	+	-	*Cronobacter sakazakii*	+	21.45	34.41	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **K**

Lactic flora: 9.4 x10^4/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: Sure Tect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
K2	-	/	/	/	-	/	32.87	-	-	-	/	-	**NA**
K6	-	/	/	/	-	/	32.15	-	-	-	/	-	**NA**
K8	-	/	/	/	-	/	34.32	-	-	-	/	-	**NA**
K11	-	/	/	/	-	/	34.89	-	-	-	/	-	**NA**
K15	-	/	/	/	-	37.07	33.41	+	-	-	/	-	**PPNA**
K19	-	/	/	/	-	/	33.52	-	-	-	/	-	**NA**
K22	-	/	/	/	-	/	33.79	-	-	-	/	-	**NA**
K23	-	/	/	/	-	42.12	32.63	+	-	-	/	-	**PPNA**
K3	+	+	-	*Cronobacter sakazakii*	+	22.33	33.03	+	+	+	*Cronobacter sakazakii*	+	**PA**
K7	+	+	-	*Cronobacter sakazakii*	+	20.61	33.84	+	+	+	*Cronobacter sakazakii*	+	**PA**
K9	-	/	/	/	-	/	34.12	-	-	-	/	-	**NA**
K13	+	+	-	*Cronobacter sakazakii*	+	21.51	32.95	+	+	+	*Cronobacter sakazakii*	+	**PA**
K16	+	+	-	*Cronobacter sakazakii*	+	20.49	33.32	+	+	+	*Cronobacter sakazakii*	+	**PA**
K18	+	+	-	*Cronobacter sakazakii*	+	20.55	33.2	+	+	+	*Cronobacter sakazakii*	+	**PA**
K21	-	/	/	/	-	39.96	34.1	-	-	-	/	-	**PPNA**
K24	+	+	-	*Cronobacter sakazakii*	+	21.08	33.61	+	+	+	*Cronobacter sakazakii*	+	**PA**
K1	+	+	-	*Cronobacter sakazakii*	+	19.8	34.82	+	+	+	*Cronobacter sakazakii*	+	**PA**
K4	+	+	-	*Cronobacter sakazakii*	+	20.44	31.19	+	+	+	*Cronobacter sakazakii*	+	**PA**
K5	+	+	-	*Cronobacter sakazakii*	+	20.84	33.19	+	+	+	*Cronobacter sakazakii*	+	**PA**
K10	+	+	-	*Cronobacter sakazakii*	+	20.01	35.36	+	+	+	*Cronobacter sakazakii*	+	**PA**
K12	+	+	-	*Cronobacter sakazakii*	+	21.23	33.05	+	+	+	*Cronobacter sakazakii*	+	**PA**
K14	+	+	-	*Cronobacter sakazakii*	+	20.83	33.94	+	+	+	*Cronobacter sakazakii*	+	**PA**
K17	+	+	-	*Cronobacter sakazakii*	+	20.32	33.42	+	+	+	*Cronobacter sakazakii*	+	**PA**
K20	+	+	-	*Cronobacter sakazakii*	+	20.44	32.31	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **L**

Lactic flora: 5.6 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
L2	-	/	/	/	-	/	33.98	-	-	-	/	-	**NA**
L6	-	/	/	/	-	/	34.02	-	-	-	/	-	**NA**
L8	-	/	/	/	-	/	34.53	-	-	-	/	-	**NA**
L11	-	/	/	/	-	/	34.1	-	-	-	/	-	**NA**
L15	-	/	/	/	-	39.16/-	34.92	+/-	-	-	/	-	**PPNA**
L19	-	/	/	/	-	40.78/-	33.46	+/-	-	-	/	-	**PPNA**
L22	-	/	/	/	-	/	33.61	-	-	-	/	-	**NA**
L23	-	/	/	/	-	40.75/-	34.19	+/-	-	-	/	-	**PPNA**
L3	+	+	-	*Cronobacter sakazakii*	+	21.29	33.69	+	+	/	*Cronobacter sakazakii*	+	**PA**
L7	+	+	-	*Cronobacter sakazakii*	+	23.52	34.29	+	+	/	*Cronobacter sakazakii*	+	**PA**
L9	+	+	-	*Cronobacter sakazakii*	+	21.06	34.29	+	+	/	*Cronobacter sakazakii*	+	**PA**
L13	+	+	-	*Cronobacter sakazakii*	+	19.91	33.39	+	+	/	*Cronobacter sakazakii*	+	**PA**
L16	+	+	-	*Cronobacter sakazakii*	+	19.87	33.03	+	+	/	*Cronobacter sakazakii*	+	**PA**
L18	+	+	-	*Cronobacter sakazakii*	+	21.9	33.46	+	+	/	*Cronobacter sakazakii*	+	**PA**
L21	-	/	/	/	-	/	33.53	-	-	-	/	-	**NA**
L24	-	/	/	/	-	/	35.04	-	-	-	/	-	**NA**
L1	+	+	-	*Cronobacter sakazakii*	+	22.1	35.28	+	+	/	*Cronobacter sakazakii*	+	**PA**
L4	+	+	-	*Cronobacter sakazakii*	+	22.17	34.39	+	+	/	*Cronobacter sakazakii*	+	**PA**
L5	+	+	-	*Cronobacter sakazakii*	+	21.19	32.53	+	+	/	*Cronobacter sakazakii*	+	**PA**
L10	+	+	-	*Cronobacter sakazakii*	+	21.3	34.52	+	+	/	*Cronobacter sakazakii*	+	**PA**
L12	+	+	-	*Cronobacter sakazakii*	+	20.75	33.78	+	+	/	*Cronobacter sakazakii*	+	**PA**
L14	+	+	-	*Cronobacter sakazakii*	+	21.89	34.8	+	+	/	*Cronobacter sakazakii*	+	**PA**
L17	+	+	-	*Cronobacter sakazakii*	+	21.63	35.59	+	+	/	*Cronobacter sakazakii*	+	**PA**
L20	+	+	-	*Cronobacter sakazakii*	+	21.46	33.95	+	+	/	*Cronobacter sakazakii*	+	**PA**

Laboratory **M**

Lactic flora: 1.5 x10^5/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/CCI Agar	Biochemical galleries		
M2	-	/	/	/	-	/	34.08	-	-	-	/	-	**NA**
M6	-	/	/	/	-	/	35.29	-	-	-	/	-	**NA**
M8	-	/	/	/	-	/	34.16	-	-	-	/	-	**NA**
M11	-	/	/	/	-	/	36.04	-	-	-	/	-	**NA**
M15	-	/	/	/	-	/	34.01	-	-	-	/	-	**NA**
M19	-	/	/	/	-	/	33.95	-	-	-	/	-	**NA**
M22	-	/	/	/	-	/	33.7	-	-	-	/	-	**NA**
M23	-	/	/	/	-	/	36.14	-	-	-	/	-	**NA**
M3	+	+	-	*Cronobacter sakazakii*	+	21.83	34.09	+	+	+	*Cronobacter sakazakii*	+	**PA**
M7	-	/	/	/	-	/	35.77	-	-	-	/	-	**NA**
M9	+	+	-	*Cronobacter sakazakii*	+	21.27	34.6	+	+	+	*Cronobacter sakazakii*	+	**PA**
M13	+	+	-	*Cronobacter sakazakii*	+	20.03	33.72	+	+	+	*Cronobacter sakazakii*	+	**PA**
M16	-	/	/	/	-	/	34.94	-	-	-	/	-	**NA**
M18	+	+	-	*Cronobacter sakazakii*	+	23.33	35.12	+	+	+	*Cronobacter sakazakii*	+	**PA**
M21	+	+	-	*Cronobacter sakazakii*	+	21.88	33.83	+	+	+	*Cronobacter sakazakii*	+	**PA**
M24	+	+	-	*Cronobacter sakazakii*	+	22.88	35.52	+	+	+	*Cronobacter sakazakii*	+	**PA**
M1	+	+	-	*Cronobacter sakazakii*	+	20.08	33.12	+	+	+	*Cronobacter sakazakii*	+	**PA**
M4	+	+	-	*Cronobacter sakazakii*	+	21.1	32.07	+	+	+	*Cronobacter sakazakii*	+	**PA**
M5	+	+	-	*Cronobacter sakazakii*	+	20.09	32.74	+	+	+	*Cronobacter sakazakii*	+	**PA**
M10	+	+	-	*Cronobacter sakazakii*	+	20.01	33.57	+	+	+	*Cronobacter sakazakii*	+	**PA**
M12	+	+	-	*Cronobacter sakazakii*	+	21.17	33.7	+	+	+	*Cronobacter sakazakii*	+	**PA**
M14	+	+	-	*Cronobacter sakazakii*	+	21.92	34.81	+	+	+	*Cronobacter sakazakii*	+	**PA**
M17	+	+	-	*Cronobacter sakazakii*	+	20.24	35.13	+	+	+	*Cronobacter sakazakii*	+	**PA**
M20	+	+	-	*Cronobacter sakazakii*	+	20.12	33.31	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **N**

Lactic flora: 5.2 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: Sure Tect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/CCI Agar	Biochemical galleries		
N2	-	/	/	/	-	35.82	33.51	+	-	-	/	-	**PPNA**
N6	-	/	/	/	-	43.66	33.24	-	-	-	/	-	**NA**
N8	-	/	/	/	-	33.22	35.16	+	-	-	/	-	**PPNA**
N11	-	/	/	/	-	/	34,00	-	-	-	/	-	**NA**
N15	-	/	/	/	-	/	33.44	-	-	-	/	-	**NA**
N19	-	/	/	/	-	/	34.26	-	-	-	/	-	**NA**
N22	-	/	/	/	-	/	34.52	-	-	-	/	-	**NA**
N23	-	/	/	/	-	/	33.6	-	-	-	/	-	**NA**
N3	+	+	-	*Cronobacter sakazakii*	+	21.83	34.6	+	+	+	*Cronobacter sakazakii*	+	**PA**
N7	+	+	-	*Cronobacter sakazakii*	+	21.79	34.58	+	+	+	*Cronobacter sakazakii*	+	**PA**
N9	-	/	/	/	-	/	33.45	-	-	-	/	-	**NA**
N13	+	+	-	*Cronobacter sakazakii*	+	19.78	34.08	+	+	+	*Cronobacter sakazakii*	+	**PA**
N16	+	+	-	*Cronobacter sakazakii*	+	19.76	35.84	+	+	+	*Cronobacter sakazakii*	+	**PA**
N18	+	+	-	*Cronobacter sakazakii*	+	42.8	32.98	-	-	-	/	-	**ND**
N21	-	/	/	/	-	/	33.51	-	-	-	/	-	**NA**
N24	-	/	/	/	-	/	34.91	-	-	-	/	-	**NA**
N1	+	+	-	*Cronobacter sakazakii*	+	20.06	34.52	+	+	+	*Cronobacter sakazakii*	+	**PA**
N4	+	+	-	*Cronobacter sakazakii*	+	20.32	34.49	+	+	+	*Cronobacter sakazakii*	+	**PA**
N5	+	+	-	*Cronobacter sakazakii*	+	20.03	33.22	+	+	+	*Cronobacter sakazakii*	+	**PA**
N10	+	+	-	*Cronobacter sakazakii*	+	19.81	34.56	+	+	+	*Cronobacter sakazakii*	+	**PA**
N12	+	+	-	*Cronobacter sakazakii*	+	20.14	33.27	+	+	+	*Cronobacter sakazakii*	+	**PA**
N14	+	+	-	*Cronobacter sakazakii*	+	20.12	33.35	+	+	+	*Cronobacter sakazakii*	+	**PA**
N17	+	+	-	*Cronobacter sakazakii*	+	19.82	34.11	+	+	+	*Cronobacter sakazakii*	+	**PA**
N20	+	+	-	*Cronobacter sakazakii*	+	20.35	35.16	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **O**

Lactic flora: 1.2 x10^5/g

N°Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/CCI Agar	Biochemical galleries		
O2	-	/	/	/	-	/	33.16	-	-	-	/	-	**NA**
O6	-	/	/	/	-	/	33.94	-	-	-	/	-	**NA**
O8	-	/	/	/	-	/	35.12	-	-	-	/	-	**NA**
O11	-	/	/	/	-	/	34.21	-	-	-	/	-	**NA**
O15	-	/	/	/	-	/	32.28	-	-	-	/	-	**NA**
O19	-	/	/	/	-	/	33.66	-	-	-	/	-	**NA**
O22	-	/	/	/	-	/	35.03	-	-	-	/	-	**NA**
O23	-	/	/	/	-	/	34.02	-	-	-	/	-	**NA**
O3	+	+	-	*Cronobacter sakazakii*	+	20.85	33.57	+	+	+	*Cronobacter sakazakii*	+	**PA**
O7	+	+	-	*Cronobacter sakazakii*	+	24.04	34.68	+	+	+	*Cronobacter sakazakii*	+	**PA**
O9	-	/	/	/	-	/	34.16	-	-	-	/	-	**NA**
O13	-	/	/	/	-	/	31.98	-	-	-	/	-	**NA**
O16	+	+	-	*Cronobacter sakazakii*	+	21.46	34.79	+	+	+	*Cronobacter sakazakii*	+	**PA**
O18	+	+	-	*Cronobacter sakazakii*	+	24.84	32.51	+	+	+	*Cronobacter sakazakii*	+	**PA**
O21	+	+	-	*Cronobacter sakazakii*	+	21.49	34.48	+	+	+	*Cronobacter sakazakii*	+	**PA**
O24	+	+	-	*Cronobacter sakazakii*	+	19.22	34.31	+	+	+	*Cronobacter sakazakii*	+	**PA**
O1	+	+	-	*Cronobacter sakazakii*	+	20.21	34.46	+	+	+	*Cronobacter sakazakii*	+	**PA**
O4	+	+	-	*Cronobacter sakazakii*	+	20.23	34.97	+	+	+	*Cronobacter sakazakii*	+	**PA**
O5	+	+	-	*Cronobacter sakazakii*	+	20.56	33.27	+	+	+	*Cronobacter sakazakii*	+	**PA**
O10	+	+	-	*Cronobacter sakazakii*	+	20.23	33.32	+	+	+	*Cronobacter sakazakii*	+	**PA**
O12	+	+	-	*Cronobacter sakazakii*	+	20.79	33.64	+	+	+	*Cronobacter sakazakii*	+	**PA**
O14	+	+	-	*Cronobacter sakazakii*	+	19.99	33.87	+	+	+	*Cronobacter sakazakii*	+	**PA**
O17	+	+	-	*Cronobacter sakazakii*	+	20.04	33.21	+	+	+	*Cronobacter sakazakii*	+	**PA**
O20	+	+	-	*Cronobacter sakazakii*	+	20.77	34.58	+	+	+	*Cronobacter sakazakii*	+	**PA**

Laboratory **P (ADRIA)**

Lacticc flora: 8.5 x10^4/g

N° Sample	Reference method: ISO/TS 22964					Alternative method: SureTect Cronobacter species PCR Assay							Agreement
						PCR			Confirmation			Final result	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Cq target	Cq IAC	Test result	CCI Agar	CSB/ CCI Agar	Biochemical galleries		
P2	-	/	/	/	-	/	33.66	-	-	-	/	-	**NA**
P6	-	/	/	/	-	/	33.69	-	-	-	/	-	**NA**
P8	-	/	/	/	-	/	35.11	-	-	-	/	-	**NA**
P11	-	/	/	/	-	/	33.89	-	-	-	/	-	**NA**
P15	-	/	/	/	-	/	34.04	-	-	-	/	-	**NA**
P19	-	/	/	/	-	/	34.35	-	-	-	/	-	**NA**
P22	-	/	/	/	-	/	33.37	-	-	-	/	-	**NA**
P23	-	/	/	/	-	/	34.34	-	-	-	/	-	**NA**
P3	-	/	/	/	-	/	34.97	-	-	-	/	-	**NA**
P7	-	/	/	/	-	/	33.44	-	-	-	/	-	**NA**
P9	+	+	+	*Cronobacter sakazakii*	+	20.36	35.39	+	+	+	*Cronobacter sakazakii*	+	**PA**
P13	-	/	/	/	-	/	33.91	-	-	-	/	-	**NA**
P16	-	/	/	/	-	/	34.28	-	-	-	/	-	**NA**
P18	-	/	/	/	-	/	34.05	-	-	-	/	-	**NA**
P21	+	+	+	*Cronobacter sakazakii*	+	22.05	33.9	+	+	+	*Cronobacter sakazakii*	+	**PA**
P24	-	/	/	/	-	/	34.38	-	-	-	/	-	**NA**
P1	+	+	+	*Cronobacter sakazakii*	+	19.85	34.64	+	+	+	*Cronobacter sakazakii*	+	**PA**
P4	+	+	+	*Cronobacter sakazakii*	+	20.39	34.51	+	+	+	*Cronobacter sakazakii*	+	**PA**
P5	+	+	+	*Cronobacter sakazakii*	+	20.23	32.2	+	+	+	*Cronobacter sakazakii*	+	**PA**
P10	+	+	+	*Cronobacter sakazakii*	+	21.31	34.34	+	+	+	*Cronobacter sakazakii*	+	**PA**
P12	+	+	+	*Cronobacter sakazakii*	+	20.72	34.44	+	+	+	*Cronobacter sakazakii*	+	**PA**
P14	+	+	+	*Cronobacter sakazakii*	+	20.79	33.85	+	+	+	*Cronobacter sakazakii*	+	**PA**
P17	+	+	+	*Cronobacter sakazakii*	+	20.27	34.15	+	+	+	*Cronobacter sakazakii*	+	**PA**
P20	+	+	+	*Cronobacter sakazakii*	+	20.51	32.9	+	+	+	*Cronobacter sakazakii*	+	**PA**

Appendix H

New protocol of the alternative method: Thermo Scientific™ SureTect™ *Cronobacter* species PCR Assay for the detection of *Cronobacter spp*

Infant formula, infant cereals, related ingredients (1)

375 g sample size

375 g + 1875 ml BPW
(+ novobiocin at 6 mg/L for PIF with probiotics)

↓

18-24 h at 34-38°C

Environmental samples (2)

25 g or surface sample

BPW

↓

20-28 h at 34-38°C

Possibility to store for 72 h at 5°C ± 3°C

Transfer an aliquot in a sterile tube

↓

In a Lysis Reagent 1 tube, add:
- 10 µl Proteinase K
- 10 µl Lysis Reagent 2
- 10 µl aliquoted enriched sample

Close with convex caps, using a Thermo Scientific™ CapEase™ tool

↓

Heat treatment in a heating block

↓

37°C for 10 min
95°C for 5 min

↓

Cool 2 min at room temperature

↓

Place SureTect™ PCR tubes at room temperature (5 min)

Run PCR on 20 µl of lysate
- Take care not to disrupt the particles at the bottom of the Lysis Tubes to ensure that no particles are transferred into the PCR Tubes
- Take care not to touch the pellet when adding the lysate
- Close with flat caps, by hand

Vortex the PCR tubes in order to dissolve the pellet in the lysate for 10 sec

↓

Positive results confirmed by:

BPW subculture in CSB broth (0.1 ml + 10 ml)

↓

24 h ± 2 h at 41.5°C ± 1°C

↓

Streaking (10 µl) onto CCI Agar

↓

224 h ± 2 h at 41.5°C ± 1°C

OR

BWP streaking (10 µl) onto CCI Agar

↓

24 h ± 2 h at 41.5°C ± 1°C

↓

Typical colonies (blue green)

↓

Biochemical tests*

*According to ISO rules, it as well possible to use ISO 16140-6:2019 validated confirmation method.

(1) According ISO 6887 requirements
(2) According ISO 18593 requirements

For sampling after cleaning process premoisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml BPW)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml BPW)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml BPW)

Appendix I – Flow diagram of the reference method: ISO 22964 (April 2017) - Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

Infant formula and ingredients:

10 g + 90 mL BPW

Environmental samples[2]:

1 swab + 10 mL BPW [2]

or 1 sponge + 100 mL BPW [2]

or 10 g + 90 mL BPW[2]

or 10 mL

↓

Incubation 18 h ± 2 h at 34 – 38°C

↓

0.1 mL + 10 mL CSB

↓

Incubation 24 h ± 2 h at 41.5°C ± 1°C

↓

Streaking onto CCI Agar plate

↓

Incubation 24 h ± 2 h at 41.5°C ± 1°C

↓

Confirmatory test on one typical colony, and four other colonies

(if the first one is negative (typical colony: blue to blue-green))

Streaking onto TSA

↓

Incubation 18 h – 24 h at 37°C ± 1°C

↓

Biochemical confirmation (oxidase, mini-galleries)

---

[3] For sampling after cleaning process premoisten
- 1 swab + 1 ml broth universal neutralizing (+ 9 ml BPW)
- 1 sponge + 10 ml broth universal neutralizing (+ 90 ml BPW)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 ml BPW)

Appendix J - Artificial contaminations

Type	#	Sample	Probiotics	Strain	Reference	Origin	Injury protocol	Injury measurement	Inoc. level CFU/sample	Result
a-	2483217	Organic infant formula 2nd age	*Limosilactobacillus fermentum heredium* 1.1 10^7 CFU/g	*C. turincensis*	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.6	-
a-	2428653	Cereals with cocoa	*B.lactis 3,4* 10^5 *CFU/g*	*C. sakazakii*	XBG933	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.7	-
a-	2428654	Cereals with biscuit	*B.lactis 3,6* 10^5 *CFU/g*	*C. sakazakii*	HZP119	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.9	-
a-	2428656	Cereals with rice	*B.lactis 1* 10^7 *CFU/g*	*C. malonaticus*	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.6	-
a+	2483211	Infant formula 1st age	*Bifidobacterium lactis* 1.4 10^7 CFU/g	*C. sakazakii*	JCT201	Milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2483212	Infant formula 1st age	*S.thermophilus* 7.7 10^6 CFU/g	*C. turincensis*	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2483213	Infant formula 1st age	*Bifidobacterium infantis* 6.3 10^6 CFU/g	*C. sakazakii*	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
a+	2483214	Infant formula 1st age	*Bifidobacterium lactis* 5.1 10^7 CFU/g	*C. dublinensis*	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.6	+
a+	2483215	Infant formula 2nd age	*Lactobacillus reuteri DSM17938* 7.7 10^6 CFU/g	*C. sakazakii*	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.8	+
a+	2483216	Infant formula 2nd age	*Bifidobacterium infantis* 5.8 10^6 CFU/g	*C. sakazakii*	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2483218	Infant formula 2nd age	*Bifidobacterium lactis* 8.6 10^5 CFU/g	*C. sakazakii*	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
a+	2483219	Infant formula 3rd age	*Lactobacillus reuteri* DSM17938 1.1 10^7 CFU/g	*C. dublinensis*	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
a+	2483220	Infant formula 3rd age	*Lactobacillus fermentum heredium* CECT5716 1.10^6	*C. sakazakii*	JCT201	Milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2428655	Cereals with quinoa, banana, prun	*B.lactis 3,4* 10^5 *CFU/g*	*C. sakazakii*	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.1	+
a+	2498700	Cereals with quinoa, banana, prun	*B.lactis 3,4* 10^5 *CFU/g*	*C. sakazakii*	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
b-	2428658	Organic infant formula 3rd age	/	*C. sakazakii*	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.8	-
b-	2428659	PIF 1st age batch 1	/	*C. turincensis*	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.2	-
b-	2428661	PIF baby croissance batch 1	/	*C. dublinensis*	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.3	-
b-	2428662	PIF baby croissance batch 2	/	*C. dublinensis*	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.6	-
b-	2428664	Infant cereals biscuit	/	*C. sakazakii*	XBG933	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.7	-
b-	2428665	Infant cereals cocoa & wheat	/	*C. sakazakii*	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.1	-
b-	2428666	Infant cereals vanilla	/	*C. sakazakii*	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.1	-
b-	2428667	Infant cereals honey	/	*C. sakazakii*	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.7	-
b+	2428660	PIF 1st age batch 2	/	*C. sakazakii*	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.3	+
b+	2428663	Infant cereals brioche - cocoa	/	*C. muytjensi*	GVW884	Collection strain	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.5	+
b+	2428668	Infant cereals vanilla	/	*C. sakazakii*	HXA137	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
b+	2498707	Infant cereals cocoa & wheat	/	*C. sakazakii*	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
b+	2498708	Infant formula for 6 at 12 months old	/	*C. sakazakii*	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
b+	2498730	Infant formula for 1 at 3 years old batch 1	/	*C. dublinensis*	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
b+	2498731	Infant formula for 1 at 3 years old batch 2	/	*C. sakazakii*	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
b+	2498732	Infant cereals biscuit	/	*C. sakazakii*	JCT201	Milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	3.0	+
b+	2498733	Infant formula for 6 at 12 months old	/	*C. sakazakii*	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
c-	2392612	Whole milk powder	/	*C. turincensis*	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	3.0	-
c-	2392617	Lactose powder	/	*C. sakazakii*	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.2	-
c-	2392618	Soy lecithin	/	*C. malonaticus*	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	-
c-	2483274	Powdered whole milk	/	*C. turincensis*	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	-
c+	2392610	Rice flour	/	*C. dublinensis*	DSEL33	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
c+	2392613	Rye flour	/	*C. dublinensis*	DSEL33	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
c+	2392614	Wheat flour	/	*C. malonaticus*	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	+
c+	2392615	Maltodextrin	/	*C. sakazakii*	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.8	+
c+	2392616	Whey	/	*C. sakazakii*	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.8	+
c+	2483271	Whey	/	*C. sakazakii*	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
c+	2483272	Soy lecithin	/	*C. malonaticus*	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	+
c+	2483275	Caseinate	/	*C. sakazakii*	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
c+	2483276	Skimmed milk powder	/	*C. sakazakii*	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	-
c+	2498709	Powdered whole milk	/	*C. sakazakii*	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.3	+
a-	2483254	Residue maltodextrin	/	*C. sakazakii*	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.8	-
a-	2483255	Residue dextrose	/	*C. sakazakii*	HXK792	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	-
a+	2483251	Residue PIF w/o probiotics	/	*C. sakazakii*	HXK792	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	+
a+	2483252	Residues maltodextrin	/	*C. sakazakii*	HZP119	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.6	+
a+	2483253	Residues flour	/	*C. sakazakii*	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
a+	2483261	Dusts vacuum PIF w/o probiotic industry	/	*C. sakazakii*	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
a+	2483262	Dusts vacuum milk powder industry	/	*C. sakazakii*	HXA137	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
a+	2483263	Dusts vacuum PIF industry	/	*C. dublinensis*	DSEL33	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
a+	2483264	Dust suction filter weighing powders	/	*C. sakazakii*	HXA137	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+

Appendix C - Artificial contaminations

Type	#	Sample	Probiotics	Strain	Reference	Origin	Injury protocol	Injury measurement	Inoc. level CFU/sample	Result
a+	2483265	Vacuum cleaner dust spice industry	/	*C. sakazakii*	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
b-	2483202	Rinsing water milk tank 1	/	*C. sakazakii*	HYD216	Dairy environment	Seeding 3 days at 3±2°C	/	2.0	-
b+	2483196	Process water Ligne 1 R1 KS1	/	*C. dublinensis*	DSEL33	Plant products	Seeding 3 days at 3±2°C	/	2.8	+
b+	2483197	Process water Ligne 1 R1 KS2	/	*C. sakazakii*	HZP119	Dairy environment	Seeding 3 days at 3±2°C	/	1.8	+
b+	2483198	Process water Ligne 2 R1	/	*C. turincensis*	HZN090	Dairy environment	Seeding 3 days at 3±2°C	/	2.0	+
b+	2483199	Dosing water ligne 2 R1	/	*C. sakazakii*	HXK792	Dairy environment	Seeding 3 days at 3±2°C	/	1.8	+
b+	2483200	Rinsing water ligne 1 barrell emptying	/	*C. sakazakii*	HZP119	Dairy environment	Seeding 3 days at 3±2°C	/	1.8	+
b+	2483201	Rinsing water ligne 1 potion	/	*C. malonaticus*	HSE385	Plant products	Seeding 3 days at 3±2°C	/	3.0	+
b+	2483203	Rinsing water milk tank 2	/	*C. sakazakii*	JFU886	Dairy environment	Seeding 3 days at 3±2°C	/	1.6	+
b+	2498734	Rinsing water TL+line TL-TR SAEQI	/	*C. sakazakii*	JFU886	Dairy environment	Seeding 3 days at 3±2°C	/	3.0	+
b+	2498735	Rinsing water TR-TR SEQI	/	*C. sakazakii*	JFP615	Dairy environment	Seeding 3 days at 3±2°C	/	2.8	+
b+	2498736	Rinsing water TR1 SAEQI	/	*C. sakazakii*	JAY280	Dairy environment	Seeding 3 days at 3±2°C	/	3.0	+
b+	2498737	Rinsing water TL+line TL-TR SAEQI	/	*C. sakazakii*	JFU886	Dairy environment	Seeding 3 days at 3±2°C	/	1.6	+
c-	2483188	Wipe robot carpet	/	*C. muytjensi*	GVW884	Collection strain	Spiking: 7 days pH = 4	0.5	2.2	-
c+	2483189	Wipe interior small tank before washing	/	*C. turincensis*	BTYS37	Plant products	Spiking: 7 days pH = 4 then 8 minutes at 56°C	0.5	2.2	+
c+	2483190	Wipe dairy floor	/	*C. sakazakii*	HXK792	Dairy environment	Spiking: 7 days pH = 4 then 8 minutes at 56°C	1.6	4.8	+
c+	2483191	Swab zone 1 robot sleeve	/	*C. sakazakii*	HXK792	Dairy environment	Spiking: 7 days pH = 4 then 8 minutes at 56°C	1.6	3.0	+
c+	2483192	Swab zone 2 robot sleeve	/	*C. turincensis*	BTYS37	Plant products	Spiking: 7 days pH = 4 then 8 minutes at 56°C	0.5	3.0	+
c+	2483193	Swab zone 3 robot sleeve	/	*C. malonaticus*	HSE385	Plant products	Spiking: 7 days pH = 4 then 8 minutes at 56°C	1.3	5.0	+
c+	2483194	Wipe floor milk powder	/	*C. muytjensi*	GVW884	Collection strain	Spiking: 7 days pH = 4	0.5	1.6	+
c+	2483195	Wipe milk powder bench	/	*C. sakazakii*	HXK792	Dairy environment	Spiking: 7 days pH = 4 then 8 minutes at 56°C	1.6	1.6	+
c+	2498720	Wipe foot and floor CP2	/	*C. sakazakii*	JAY280	Dairy environment	Spiking: 7 days pH = 4 then 4 minutes at 56°C	0.6	2.8	+
c+	2498726	Wipe cabinet AZOMP3	/	*C. sakazakii*	JAY280	Dairy environment	Spiking: 7 days pH = 4 then 4 minutes at 56°C	0.6	2.8	+
c+	2498727	Wipe desk legs AE ST	/	*C. sakazakii*	HZP119	Dairy environment	Spiking: 7 days pH = 4 then 6 minutes at 56°C	0.7	3.0	+
c+	2498728	Swab floor between Z5 and Z6	/	*C. sakazakii*	JFP615	Dairy environment	Spiking: 7 days pH = 4 then 6 minutes at 56°C	0.6	2.4	+
c+	2498729	Swab HH wardrobe door frame	/	*C. sakazakii*	HZP119	Dairy environment	Spiking: 7 days pH = 4 then 4 minutes at 56°C	0.5	1.6	+

Appendix K
Sensitivity study - raw results

Bacterial burden	*Distribution of flora*
Ø: no culture	A = pure culture of suspect colonies
L = low	B = mixture with a majority of suspect colonies
M = moderate	C = mixture with a minority of suspect colonies
H = high	D = mixture with rare suspect colonies
/: not realized	E = absence of suspect colonies
	(x): x colonies characteristic of Cronobacter if x ≤ 5

PA: positive agreement
NA: negative agreement
ND: negative deviation
PD: positive deviation
PPNA: positive presumptive negative agreement
PPND : positive presumptive negative deviation
/: not realized

Infant formula 375 g

							Reference method				SureTect Cronobacter : pre-warmed BPW 1/6													BPW								
							ISO 22964*				18h at 34-38°C													72h at 2-8°C								
Type	#	Sample	Probiotics	Contamination			CCI After CSB	Confirmatory tests		Final result	PCR result (Cq)		Confirmation				Final result		Agreement		ISO conf.			PCR result (Cq)		Confirmation			Final result		Agreement	
				Strain		Level CFU		OX	Gallery		7500 Fast	QS5	CCI	Oxydase	Gallery without purification	Gallery with purification	7500 Fast	QS5	7500 Fast	QS5	CCI after CSB	Ox.	Gallery	7500 Fast	QS5	CCI	Oxydase	Gallery	7500 Fast	QS5	7500 Fast	QS5
a-	2483217	Organic infant formula 2nd age	*Limosilactobacillus fermentum heredium* 1.1 10^7 CFU/g	*C. turincensis*	HZN090	1.6	- (ø)	/	/	A	- 5x-	- 5x-	- (EM)	/	/	/	A (FN)	A (FN)	NA	NA	+ (BM)	-	*Cronobacter sp*	-	-	-(EM)	/	/	A	A	NA	NA
a-	2428653	Cereals with cocoa	*B.lactis 3,4 10^5 CFU/g*	*C. sakazakii*	XBG933	1.6	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	- (ø)	/	/	A	A	NA	NA
a-	2428654	Cereals with biscuit	*B.lactis 3,6 10^5 CFU/g*	*C. sakazakii*	HZP119	2.0	- (ø)	/	/	A	Inh./ pur: + 39.56; 1/10:-	+ 37.60 Retest : -	- (ø)	/	/	/	A (FP)	A (FP)	NA (PP)	NA (PP)	- (ø)	/	/	/	/	- (ø)	/	/	A	A	NA	NA
a-	2428656	Cereals with rice	*B.lactis 1 10^7 CFU/g*	*C. malonaticus*	HSE385	1.5	- (ø)	/	/	A	Inh./ pur: -; 1/10:-	-	- (EM)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
a-	2428599	Thickened formula infant milk for 0 at 6 months old	*Bifidobacterium lactis* 1.4 10^7 CFU/g	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
a-	2428600	Infant milk as a substitute for breastfeeding for 6 at 12 months old	*Lactobacillus reuteri DSM17938* 7.7 10^6 CFU/g	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
a-	2428601	Organic infant formula for 1 at 3 years old	*Bifidobacterium lactis* 5.1 10^7 CFU/g	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
a-	2428602	Infant cereals with milk chocolate and biscuit flavor	*Bifidobacterium lactis 3.4 10^5 CFU/g*	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
a-	2428603	Infant cereals with 5 cereals	*Bifidobacterium lactis 3.5 10^5 CFU/g*	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
a-	2428605	Infant cereals with rice	*Bifidobacterium lactis 1 10^7 CFU/g*	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
a+	2483211	Infant formula 1st age	*Bifidobacterium lactis* 1.4 10^7 CFU/g	*C. sakazakii*	JCT201	2.0	+ (AM)	-	*Cronobacter sp*	P	+ 22,28	+ 21,25	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 23,01	+ 21,03	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483212	Infant formula 1st age	*S.thermophilus* 7.7 10^6 CFU/g	*C. turincensis*	HZN090	2.0	+ (AM)	-	*Cronobacter sp*	P	+ 30,42	+ 28,79	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 30,51	+ 31,46	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483213	Infant formula 1st age	*Bifidobacterium infantis* 6.3 10^6 CFU/g	*C. sakazakii*	JGG241	2.6	+ (AM)	-	*Cronobacter sp*	P	+ 19,01	+ 18,34	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 18,75	+ 17,27	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483214	Infant formula 1st age	*Bifidobacterium lactis* 5.1 10^7 CFU/g	*C. dublinensis*	GVV828	1.6	- (ø)	/	/	A	+ 22,54	+ 22,24	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 21,16	+ 19,89	+ (AM)	-	*Cronobacter sp*	P	P	PD	PD
a+	2483215	Infant formula 2nd age	*Lactobacillus reuteri DSM17938* 7.7 10^6 CFU/g	*C. sakazakii*	HXN562	1.8	+ (AM)	-	*Cronobacter sp*	P	+ 29,31	+ 27,66	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 29,07	+ 27,99	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483216	Infant formula 2nd age	*Bifidobacterium infantis* 5.8 10^6 CFU/g	*C. sakazakii*	HYD216	2.0	- (ø)	/	/	A	+ 20,60	+ 19,16	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 21,18	+ 20,20	+ (AM)	-	*Cronobacter sp*	P	P	PD	PD
a+	2483218	Infant formula 2nd age	*Bifidobacterium lactis* 8.6 10^5 CFU/g	*C. sakazakii*	HXN562	2.4	+ (AM)	-	*Cronobacter sp*	P	+ 21,67	+ 20,13	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (BM)	-	*Cronobacter sp*	+ 21,42	+ 20,42	+ (BM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483219	Infant formula 3rd age	*Lactobacillus reuteri* DSM17938 1.1 10^7 CFU/g	*C. dublinensis*	GVV828	2.4	+ (AM)	-	*Cronobacter sp*	P	+ 24,82	+ 24,05	+ (DM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (BM)	-	*Cronobacter sp*	+ 23,91	+ 23,82	+ (CM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483220	Infant formula 3rd age	*Lactobacillus fermentum heredium* CECT5716 1.10^6 CFU/g	*C. sakazakii*	JCT201	2.0	+ (AM)	-	*Cronobacter sp*	P	+ 19,98	+ 18,11	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (BM)	-	*Cronobacter sp*	+ 20,22	+ 17,90	+ (BM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2428655	Cereals with quinoa, banana, prun	*B.lactis 3,4 10^5 CFU/g*	*C. sakazakii*	RLP329	2.0	- (ø)	/	/	A	+ 28.32	+ 21.03	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 23.31	+ 20.58	+ (AM)	-	*Cronobacter sp*	P	P	PD	PD
a+	2498700	Cereals with quinoa, banana, prun	*B.lactis 3,4 10^5 CFU/g*	*C. sakazakii*	JGG241	2.6	+ (AM)	-	*Cronobacter sp*	P	+ 34,45	+ 33,11	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 34,14	+ 30,73	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
b-	2428657	Infant formula 2nd age	/	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
b-	2428658	Organic infant formula 3rd age	/	*C. sakazakii*	HXN562	1.8	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
b-	2428659	PIF 1st age batch 1	/	*C. turincensis*	HZN090	1.2	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
b-	2428661	PIF baby croissance batch 1	/	*C. dublinensis*	GVV828	1.4	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
b-	2428662	PIF baby croissance batch 2	/	*C. dublinensis*	GVV828	1.6	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
b-	2428664	Infant cereals biscuit	/	*C. sakazakii*	XBG933	1.8	- (ø)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	/	/	/	/	/	/	/	/	/
b-	2428665	Infant cereals cocoa & wheat	/	*C. sakazakii*	RLP329	2.2	- (ø)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	/	/	/	/	/	/	/	/	/
b-	2428666	Infant cereals vanilla	/	*C. sakazakii*	HYD216	1.2	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	/	/	/	/	/	/	/	/	/
b-	2428667	Infant cereals honey	/	*C. sakazakii*	HYD216	1.8	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	/	/	/	/	/	/	/
b-	2428607	Infant formula for 0 at 6 months old batch 1	/	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	ø	/	/	A	A	NA	NA
b-	2428608	Infant formula for 6 at 12 months old	/	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	ø	/	/	A	A	NA	NA
b-	2428609	Infant formula for 0 at 6 months batch 2	/	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	ø	/	/	A	A	NA	NA
b-	2428610	Thickened formula infant milk for 6 at 12 months old	/	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	ø	/	/	A	A	NA	NA
b-	2428611	Organic infant formula for 1 at 3 years old	/	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	/	/	ø	/	/	A	A	NA	NA
b-	2428614	Infant cereals with biscuit flavor	/	/	/	/	- (ø)	/	/	A	-	-	-(EM)	/	/	/	A	A	NA	NA	-(EM)	/	/	/	/	/	/	/	/	/	/	/
b+	2428660	PIF 1st age batch 2	/	*C. sakazakii*	HXN562	2.2	+ (AM)	-	*Cronobacter sp*	P	-	-	- (ø)	/	/	/	A	A	ND	ND	- (ø)	/	/	-	-	- (ø)	/	/	A	A	ND	ND
b+	2428663	Infant cereals brioche - cocoa	/	*C. muytjensi*	GVW884	2.5	- (EM)	/	/	A	+ 27,94	+ 25.21	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (BM)	-	*Cronobacter sp*	+ 27.11	+ 24.34	+ (BM)	-	*Cronobacter sp*	P	P	PD	PD
b+	2428668	Infant cereals vanilla	/	*C. sakazakii*	HXA137	2.2	+ (AM)	-	*Cronobacter sp*	P	+ 30.38	+ 27.78	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (BM)	-	*Cronobacter sp*	+ 30.49	+ 26.65	+ (BM)	-	*Cronobacter sp*	P	P	PA	PA
b+	2498707	Infant cereals cocoa & wheat	/	*C. sakazakii*	JGG241	2.6	- (ø)	/	/	A	+ 34,20	+ 29,56	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 33,63	+ 30,72	+ (BM)	-	*Cronobacter sp*	P	P	PD	PD

Infant formula 375 g

							Reference method				SureTect Cronobacter : pre-warmed BPW 1/6													BPW								
							ISO 22964*				18h at 34-38°C													72h at 2-8°C								
b+	2498708	Infant formula for 6 at 12 months old	/	*C. sakazakii*	HXN562	2.0	+ (AM)	-	*Cronobacter sp*	**P**	-	-	- (ø)	/	/	/	**A**	**A**	**ND**	**ND**	- (ø)	/	/	-	-	-(EL)	/	/	**A**	**A**	**ND**	**ND**
b+	2498730	Infant formula for 1 at 3 years old batch 1	/	*C. dublinensis*	GVV828	2.4	- (ø)	/	/	**A**	+ 38,12	+ 34,93	+ (AL)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PD**	**PD**	+ (AM)	-	*Cronobacter sp*	+ 38,32	+ 34,34	+ (AL)	-	*Cronobacter sp*	**P**	**P**	**PD**	**PD**
b+	2498731	Infant formula for 1 at 3 years old batch 2	/	*C. sakazakii*	HXN562	2.4	- (ø)	/	/	**A**	+ 38,30	+ 35,39	+ (AL)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PD**	**PD**	+ (AM)	-	*Cronobacter sp*	+ 39,79	+ 37,46	+ (AL)	-	*Cronobacter sp*	**P**	**P**	**PD**	**PD**
b+	2498732	Infant cereals biscuit	/	*C. sakazakii*	JCT201	3.0	+ (AM)	-	*Cronobacter sp*	**P**	Inh./ pur: inh; 1/10:+ 28,37	+ 23,87	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 27,45	+ 24,44	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
b+	2498733	Infant formula for 6 at 12 months old	/	*C. sakazakii*	JGG241	2.6	+ (AM)	-	*Cronobacter sp*	**P**	+ 37,49	+ 33,56	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 35,23	+ 32,95	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c-	2392608	Whey permeate	/	/	/	/	- (ø)	/	/	**A**	-	-	-(EM)	/	/	/	**A**	**A**	**NA**	**NA**	-(EM)	/	/	/	/	/	/	/	**/**	**/**	**/**	**/**
c-	2392609	Corn starch	/	/	/	/	- (ø)	/	/	**A**	-	-	-(EM)	/	/	/	**A**	**A**	**NA**	**NA**	-(EM)	/	/	/	/	/	/	/	**/**	**/**	**/**	**/**
c-	2392612	Whole milk powder	/	*C. turincensis*	HZN090	3.0	- (ø)	/	/	**A**	-	-	-(EM)	/	/	/	**A**	**A**	**NA**	**NA**	-(EM)	/	/	/	/	/	/	/	**/**	**/**	**/**	**/**
c-	2392617	Lactose powder	/	*C. sakazakii*	HYD216	1.2	- (ø)	/	/	**A**	- -/-/-/+ 37.42	- -/+ 34.94/-/-	+ (AL) Bis: +AL(5)	-	*Cronobacter sp*	*Cronobacter sp*	**A (FN)**	**A (FN)**	**NA**	**NA**	+ (AM)	-	*Cronobacter sp*	- -/-/-	- -/+ 34.94/-	+ (AL)	-	*Cronobacter sp*	**A (FN)**	**A (FN)**	**NA**	**NA**
c-	2392618	Soy lecithin	/	*C. malonaticus*	HSE385	2.4	- (ø)	/	/	**A**	+ 39.69 Retest: -	+ 38.49 Retest: -	-(EM) Bis:-(EM)	/	/	/	**A (FP)**	**A (FP)**	**NA (PP)**	**NA (PP)**	-(EM)	/	/	-	-	-(EM)	/	/	**A**	**A**	**NA**	**NA**
c-	2428617	Lactose powder	/	/	/	/	- (ø)	/	/	**A**	-	-	- (ø)	/	/	/	**A**	**A**	**NA**	**NA**	- (ø)	/	/	/	/	ø	/	/	**A**	**A**	**NA**	**NA**
c-	2428618	Whey permeate	/	/	/	/	- (ø)	/	/	**A**	-	-	- (ø)	/	/	/	**A**	**A**	**NA**	**NA**	- (ø)	/	/	/	/	ø	/	/	**A**	**A**	**NA**	**NA**
c-	2428620	Powdered whole milk	/	/	/	/	- (ø)	/	/	**A**	-	-	- (ø)	/	/	/	**A**	**A**	**NA**	**NA**	- (ø)	/	/	/	/	ø	/	/	**A**	**A**	**NA**	**NA**
c-	2428621	Powdered buttermilk	/	/	/	/	- (ø)	/	/	**A**	-	-	- (ø)	/	/	/	**A**	**A**	**NA**	**NA**	- (ø)	/	/	/	/	ø	/	/	**A**	**A**	**NA**	**NA**
c-	2483274	Powdered whole milk	/	*C. turincensis*	HZN090	2.4	- (ø)	/	/	**A**	-	-	-(EL)	/	/	/	**A**	**A**	**NA**	**NA**	- (ø)	/	/	/	/	ø	/	/	**A**	**A**	**NA**	**NA**
c+	2483276	Skimmed milk powder	/	*C. sakazakii*	HYD216	2.0	- (ø)	/	/	**A**	+37.07	+35.47	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PD**	**PD**	+ (AM)	-	*Cronobacter sp*	+ 39.80	+ 33.47	+ (CL)	-	*Cronobacter sp*	**P**	**P**	**PD**	**PD**
c+	2392610	Rice flour	/	*C. dublinensis*	DSEL33	3.0	+ (AM)	-	*Cronobacter sp*	**P**	+ 25.65	+ 21.56	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (BM)	-	*Cronobacter sp*	+ 24.65	+ 21.88	+ (BM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2392613	Rye flour	/	*C. dublinensis*	DSEL33	3.0	+ (CM)	-	*Cronobacter sp*	**P**	+ 32.40	+ 27.79	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (CM)	-	*Cronobacter sp*	+ 38.48	+ 30.88	-(EM) CSB: +(BM)	/	/	**P**	**P**	**PA**	**PA**
c+	2392614	Wheat flour	/	*C. malonaticus*	HSE385	2.4	- (EM)	/	/	**A**	+ 32.89	+ 28.02	+ (DM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PD**	**PD**	+ (CM)	-	*Cronobacter sp*	+ 30.28	+ 27.47	+ (DM)	-	*Cronobacter sp*	**P**	**P**	**PD**	**PD**
c+	2392615	Maltodextrin	/	*C. sakazakii*	JBU888	1.8	- (ø)	/	/	**A**	+ 25.39	+ 20.60	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PD**	**PD**	+ (AM)	-	*Cronobacter sp*	+ 24.56	+ 20.80	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PD**	**PD**
c+	2392616	Whey	/	*C. sakazakii*	JBU888	1.8	+ (AM)	-	*Cronobacter sp*	**P**	-	-	-(EM)	/	/	/	**A**	**A**	**ND**	**ND**	- (ø)	/	/	-	-	-(EM)	/	/	**A**	**A**	**ND**	**ND**
c+	2483271	Whey	/	*C. sakazakii*	JBU888	2.2	+ (AM)	-	*Cronobacter sp*	**P**	+ 25.26	+ 20.52	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 26.28	+ 21.72	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2483272	Soy lecithin	/	*C. malonaticus*	HSE385	2.4	+ (AM)	-	*Cronobacter sp*	**P**	+37.64 +38.21/+39.02/+37.07	+38.11 +34.74/+37.36/+34.76	-(EM) Bis:-(EM)	/	/	/	**P**	**P**	**PA**	**PA**	+ (BM)	-	*Cronobacter sp*	+ 38.66	+ 36.48	-(EM) CSB: +(BM)	/	/	**P**	**P**	**PA**	**PA**
c+	2483275	Caseinate	/	*C. sakazakii*	JBU888	2.2	+ (AM)	-	*Cronobacter sp*	**P**	+ 39.69	+ 35.80	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 38.74	+ 34.27	+ (CM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2498709	Powdered whole milk	/	*C. sakazakii*	JGG241	1.3	- (ø)	/	/	**A**	+ 38,98	+ 35,02	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PD**	**PD**	+ (AM)	-	*Cronobacter sp*	+ 39,90	+ 35,01	+ (BL)	-	*Cronobacter sp*	**P**	**P**	**PD**	**PD**

Environmental samples

						Reference method				SureTect Cronobacter: BPW													BPW								
						ISO 22964*				20h at 34-38°C													72h at 2-8°C								
Type	#	Sample	Contamination			CCI After CSB	Confirmatory tests		Final result	PCR result (Cq)		Confirmation				Final result		Agreement		ISO conf.			PCR result (Cq)		Confirmation			Final result		Agreement	
			Strain		Level CFU		OX	Gallery		7500 Fast	QS5	CCI	Oxydase	Gallery without purification	Gallery with purification	7500 Fast	QS5	7500 Fast	QS5	CCI after CSB	Ox.	Gallery	7500 Fast	QS5	CCI	Oxydase	Gallery	7500 Fast	QS5	7500 Fast	QS5
a-	2483254	Residue maltodextrin	*C. sakazakii*	JBU888	1.8	- (ø)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
a-	2483255	Residue dextrose	*C. sakazakii*	HXK792	2.4	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
a-	2483256	Residue PIF w/o probiotics	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
a-	2483257	Residue maltodextrin	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
a-	2483258	Residues flour	/	/	/	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
a-	2483259	Residues maltodextrin	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
a-	2483260	Residue dextrose	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
a-	2483266	Dusts vacuum PIF w/o probiotic industry	/	/	/	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
a-	2483267	Dusts vacuum milk powder industry	/	/	/	- (ø)	/	/	A	-	-	- (EL)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (EL)	/	/	A	A	NA	NA
a-	2483268	Dusts vacuum PIF industry	/	/	/	- (EM)	/	/	A	-	-	- (EL)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (EL)	/	/	A	A	NA	NA
a-	2483269	Dust suction filter weighing powders	/	/	/	- (ø)	/	/	A	-	-	- (EL)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (EL)	/	/	A	A	NA	NA
a-	2483270	Vacuum cleaner dust spice industry	/	/	/	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
a+	2483251	Residue PIF w/o probiotics	*C. sakazakii*	HXK792	2.4	+ (AM)	-	*Cronobacter sp*	P	+ 32.75	+ 27.65	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 30.44	+ 26.75	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483252	Residues maltodextrin	*C. sakazakii*	HZP119	2.6	+ (AM)	-	*Cronobacter sp*	P	+ 25.17	+ 21.61	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 24.32	+ 19.87	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483253	Residues flour	*C. sakazakii*	JBU888	2.2	+ (CM)	-	*Cronobacter sp*	P	+ 34.02	+ 31.68	+ (CM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (CM)	-	*Cronobacter sp*	+ 33.81	+ 29.96	+ (CM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483261	Dusts vacuum PIF w/o probiotic industry	*C. sakazakii*	RLP329	3.0	+ (AM)	-	*Cronobacter sp*	P	+ 30.25	+ 28.08	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 30.88	+ 26.97	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483262	Dusts vacuum milk powder industry	*C. sakazakii*	HXA137	2.2	+ (AM)	-	*Cronobacter sp*	P	+ 26.97	+ 25.80	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 27.91	+ 24.57	+ (BL)	-	*Cronobacter sp*	P	P	PA	PA
a+	2483263	Dusts vacuum PIF industry	*C. dublinensis*	DSEL33	3.0	- (ø)	/	/	A	+ 28.45	+ 25.89	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 27.85	+ 23.96	+ (BM)	-	*Cronobacter sp*	P	P	PD	PD
a+	2483264	Dust suction filter weighing powders	*C. sakazakii*	HXA137	2.2	+ (AM)	-	*Cronobacter sp*	P	-	-	- (ø)	/	/	/	A	A	ND	ND	- (ø)	/	/	-	-	- (EL)	/	/	A	A	ND	ND
a+	2483265	Vacuum cleaner dust spice industry	*C. sakazakii*	RLP329	3.0	+ (AM)	-	*Cronobacter sp*	P	+ 32.27	+ 28.40	+ (CM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (BM)	-	*Cronobacter sp*	+ 31.64	+ 27.83	+ (DM)	-	*Cronobacter sp*	P	P	PA	PA
b-	2483202	Rinsing water milk tank 1	*C. sakazakii*	HYD216	2.0	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA				-	-	- (EM)	/	/	A	A	NA	NA
b-	2498691	Process water Ligne 1 R1 KS1	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498692	Process water Ligne 1 R1 KS2	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498693	Process water Ligne 2 R1	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498694	Dosing water ligne 2 R1	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498695	Rinsing water ligne 1 barrel emptying	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498696	Rinsing water ligne 1 potion	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498697	Rinsing water milk tank 1	/	/	/	- (ø)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
b-	2498698	Rinsing water milk tank 2	/	/	/	- (ø)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
b-	2498712	Rinsing water TL+line TL-TR SAEQI	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498713	Rinsing water TR-TR SEQI	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498714	Rinsing water TR1 SAEQI	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA

Environmental samples

						Reference method				SureTect Cronobacter: BPW													BPW								
						ISO 22964*				20h at 34-38°C													72h at 2-8°C								
b-	2498715	Rinsing water TL+line TL-TR SAEQI	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498716	Rinsing water TR-TR SEQI	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b-	2498717	Rinsing water TR1 SAEQI	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
b+	2483196	Process water Ligne 1 R1 KS1	*C. dublinensis*	DSEL33	2.8	+ (AM)	-	*Cronobacter sp*	P	+ 23.45	+ 18.86	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 25.19	+ 19.15	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
b+	2483197	Process water Ligne 1 R1 KS2	*C. sakazakii*	HZP119	1.8	+ (AM)	-	*Cronobacter sp*	P	+ 23.32	+ 18.79	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 24.43	+ 19.52	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
b+	2483198	Process water Ligne 2 R1	*C. turincensis*	HZN090	2.0	- (ø)	/	/	A	+ 24.48	+ 19.74	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 24.23	+ 19.53	+ (AM)	-	*Cronobacter sp*	P	P	PD	PD
b+	2483199	Dosing water ligne 2 R1	*C. sakazakii*	HXK792	1.8	+ (AM)	-	*Cronobacter sp*	P	-	-	- (ø)	/	/	/	A	A	ND	ND	- (ø)	/	/	-	-	- (ø)	/	/	A	A	ND	ND
b+	2483200	Rinsing water ligne 1 barrell emptying	*C. sakazakii*	HZP119	1.8	+ (AM)	-	*Cronobacter sp*	P	-	-	- (ø)	/	/	/	A	A	ND	ND	- (ø)	/	/	-	-	- (ø)	/	/	A	A	ND	ND
b+	2483201	Rinsing water ligne 1 potion	*C. malonaticus*	HSE385	3.0	+ (AM)	-	*Cronobacter sp*	P	+ 23.70	+ 19.45	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)			+ 25.21	+ 19.94	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
b+	2483203	Rinsing water milk tank 2	*C. sakazakii*	JFU886	1.6	+ (AM)	-	*Cronobacter sp*	P	-	-	- (EM)	/	/	/	A	A	ND	ND	- (EM)	/	/	-	-	- (EM)	/	/	A	A	ND	ND
b+	2498734	Rinsing water TL+line TL-TR SAEQI	*C. sakazakii*	JFU886	3.0	- (EM)	/	/	A	+ 26.07	+ 19.53	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PD	PD	+ (AM)	-	*Cronobacter sp*	+ 25,79	+ 20.07	+ (AM)	-	*Cronobacter sp*	P	P	PD	PD
b+	2498735	Rinsing water TR-TR SEQI	*C. sakazakii*	JFP615	2.8	+ (AM)	-	*Cronobacter sp*	P	+ 26.30	+ 21.15	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 26,34	+ 20.15	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
b+	2498736	Rinsing water TR1 SAEQI	*C. sakazakii*	JAY280	3.0	+ (AM)	-	*Cronobacter sp*	P	+ 25.47	+ 19.42	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 25,62	+ 20.38	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
b+	2498737	Rinsing water TL+line TL-TR SAEQI	*C. sakazakii*	JFU886	1.6	+ (AM)	-	*Cronobacter sp*	P	+ 24.50	+ 19.58	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 25,74	+ 20.44	+ (AM)	-	*Cronobacter sp*	P	P	PA	PA
c-	2483188	Wipe robot carpet	*C. muytjensi*	GVW884	2.2	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
c-	2498684	Wipe interior small tank before washing	/	/	/	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
c-	2498685	Wipe dairy floor	/	/	/	- (EM)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
c-	2498686	Swab zone 1 robot sleeve	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498687	Swab zone 2 robot sleeve	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498688	Swab zone 3 robot sleeve	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498689	Wipe floor milk powder	/	/	/	- (ø)	/	/	A	-	-	- (EL)	/	/	/	A	A	NA	NA	- (EM)	/	/	-	-	- (EL)	/	/	A	A	NA	NA
c-	2498690	Wipe milk powder bench	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498718	Wipe cabinet AZOMP3	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498719	Wipe desk legs AE ST	/	/	/	- (ø)	/	/	A	-	-	- (EM)	/	/	/	A	A	NA	NA	- (EL)	/	/	-	-	- (EM)	/	/	A	A	NA	NA
c-	2498721	Wipe sieve foot	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498722	Swab locker room washbasin	/	/	/	- (EL)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498723	Swab floor between Z5 and Z6	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498724	Swab siphon HP filter tray	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c-	2498725	Swab HH wardrobe door frame	/	/	/	- (ø)	/	/	A	-	-	- (ø)	/	/	/	A	A	NA	NA	- (ø)	/	/	-	-	- (ø)	/	/	A	A	NA	NA
c+	2483189	Wipe interior small tank before washing	*C. turincensis*	BTYS37	2.2	+ (BM)	-	*Cronobacter sp*	P	-	-	- (EM)	/	/	/	A	A	ND	ND	- (EM)	/	/	-	-	- (EM)	/	/	A	A	ND	ND
c+	2483190	Wipe dairy floor	*C. sakazakii*	HXK792	4.8	+ (DM)	-	*Cronobacter sp*	P	-	-	- (EM)	/	/	/	A (FN)	A (FN)	ND	ND	+ (BM)	-	*Cronobacter sp*	-	+ 36.34	- (EM) CSB: + (BM)	/	/	A	P	ND	PA
c+	2483191	Swab zone 1 robot sleeve	*C. sakazakii*	HXK792	3.0	+ (AM)	-	*Cronobacter sp*	P	+ 23.79	+ 19.89	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	P	P	PA	PA	+ (AM)	-	*Cronobacter sp*	+ 23.48	+ 18.79	+ (AL)	-	*Cronobacter sp*	P	P	PA	PA

Environmental samples

						Reference method				SureTect Cronobacter: BPW													BPW								
						ISO 22964*				20h at 34-38°C													72h at 2-8°C								
c+	2483192	Swab zone 2 robot sleeve	*C. turincensis*	BTYS37	3.0	+ (AM)	-	*Cronobacter sp*	**P**	+ 24.65	+ 19.63	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 25.10	+ 19.92	+ (AL)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2483193	Swab zone 3 robot sleeve	*C. malonaticus*	HSE385	5.0	+ (AM)	-	*Cronobacter sp*	**P**	+ 26.49	+ 21.60	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 25.59	+ 21.60	+ (AL)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2483194	Wipe floor milk powder	*C. muytjensi*	GVW884	1.6	+ (AM)	-	*Cronobacter sp*	**P**	+ 31.18	+ 26.67	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 30.57	27.05	+ (DM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2483195	Wipe milk powder bench	*C. sakazakii*	HXK792	1.6	+ (AM)	-	*Cronobacter sp*	**P**	+ 23.15	+ 18.62	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 22.20	+ 18.23	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2498720	Wipe foot and floor CP2	*C. sakazakii*	JAY280	2.8	+ (AM)	-	*Cronobacter sp*	**P**	+ 24.12	+ 18.12	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 22.18	+ 17.32	+ (BM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2498726	Wipe cabinet AZOMP3	*C. sakazakii*	JAY280	2.8	+ (AM)	-	*Cronobacter sp*	**P**	+ 23.14	+ 18.83	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 23.61	+ 18.70	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2498727	Wipe desk legs AE ST	*C. sakazakii*	HZP119	3.0	+ (AM)	-	*Cronobacter sp*	**P**	+ 25.91	+ 20.16	+ (BM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 26.09	+ 20.39	+ (BM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2498728	Swab floor between Z5 and Z6	*C. sakazakii*	JFP615	2.4	+ (AM)	-	*Cronobacter sp*	**P**	+ 24.70	+ 20.25	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 24.84	+ 18.70	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**
c+	2498729	Swab HH wardrobe door frame	*C. sakazakii*	HZP119	1.6	+ (AM)	-	*Cronobacter sp*	**P**	+ 24.33	+ 19.62	+ (AM)	-	*Cronobacter sp*	*Cronobacter sp*	**P**	**P**	**PA**	**PA**	+ (AM)	-	*Cronobacter sp*	+ 24.74	+ 19.35	+ (AM)	-	*Cronobacter sp*	**P**	**P**	**PA**	**PA**

Appendix L
Relative Level of Detection - raw results

Infant formula 375 g

Total aerobic microflora :100 UFC/g

				Reference method					SureTect Cronobacter PCR assay : pre-warmed BPW 1/6								
				ISO 22964■					18h at 34-38°C								
Sample	Strain	Product	Inoculation/level	CCI After CSB	Confirmatory tests		Final result	Number positive samples/Total	PCR result (Cq)		Confirmation				Final result		Number positive samples/Total
					OX	Gallery			7500 Fast	QS5	CCI	Oxydase	Gallery without purification	Gallery with purification	7500 Fast	QS5	
2498654	*Cronobacter sakazakii* JBU888	Infant formula with probiotic *Lactobacillus reuteri* DSM 17938 1,1 10^7 CFU/g	0	- (∅)	/	/	**A**	0/5	-	-	- (∅)	/	/	/	**A**	**A**	0/5
2498655				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498656				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498657				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498658				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498659			1.0	- (∅)	/	/	**A**	6/20	-	-	- (∅)	/	/	/	**A**	**A**	3/20
2498660				- (∅)	/	/	**A**		+ 38,17	+ 36,25	+ (AL)	-	*Cronobacter sp.*	*Cronobacter sp.*	**P**	**P**	
2498661				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498662				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498663				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498664				+ (AM)	-	*Cronobacter sp.*	**P**		-	-	- (∅)	/	/	/	**A**	**A**	
2498665				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498666				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498667				+ (AM)	-	*Cronobacter sp.*	**P**		-	-	- (∅)	/	/	/	**A**	**A**	
2498668				+ (AM)	-	*Cronobacter sp.*	**P**		-	-	- (∅)	/	/	/	**A**	**A**	
2498669				+ (AM)	-	*Cronobacter sp.*	**P**		-	-	- (∅)	/	/	/	**A**	**A**	
2498670				- (∅)	/	/	**A**		-/-	-/-	+ (AL) 4	-	*Cronobacter sp.*	*Cronobacter sp.*	**A (FN)**	**A (FN)**	
2498671				- (∅)	/	/	**A**		+ 37,82	+ 32,17	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	**P**	**P**	
2498672				- (∅)	/	/	**A**		+ 32,81	+ 28,53	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	**P**	**P**	
2498673				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498674				+ (AM)	-	*Cronobacter sp.*	**P**		-	-	- (∅)	/	/	/	**A**	**A**	
2498675				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498676				+ (AM)	-	*Cronobacter sp.*	**P**		-	-	- (∅)	/	/	/	**A**	**A**	
2498677				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498678				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498679			2.7	- (∅)	/	/	**A**	1/5	-	-	- (∅)	/	/	/	**A**	**A**	1/5
2498680				+ (AM)	-	*Cronobacter sp.*	**P**		+ 31,90	+ 29,66	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	**P**	**P**	
2498681				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498682				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	
2498683				- (∅)	/	/	**A**		-	-	- (∅)	/	/	/	**A**	**A**	

Environmental sample

Total aerobic microflora :200 UFC/surface

				Reference method					BPW								
				ISO 22964■					20h at 34-38°C								
					Confirmatory tests				PCR result (Cq)		Confirmation				Final result		
Sample	Strain	Product	Inoculation /level	CCI After CSB	OX	Gallery	Final result	Number positive samples/Total	7500 Fast	QS5	CCI	Oxydase	Gallery without purification	Gallery with purification	7500 Fast	QS5	Number positive samples/Total
2483221	*Cronobacter sakazakii* HZN090	Stainless steel 1"x1"	0	- (∅)	/	/	A	0/5	-	-	- (∅)	/	/	/	A	A	0/5
2483222				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483223				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483224				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483225				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483226			9100	+ (AM)	-	*Cronobacter sp.*	P	5/20	-	-	- (∅)	/	/	/	A	A	5/20
2483227				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483228				+ (AM)	-	*Cronobacter sp.*	P		+ 22.19	+ 18.47	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483229				- (∅)	/	/	A		+ 23.90	+ 20.59	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483230				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483231				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483232				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483233				- (∅)	/	/	A		+ 23.77	+ 19.14	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483234				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483235				- (∅)	/	/	A		+ 25.43	+ 18.94	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483236				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483237				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483238				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483239				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483240				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483241				+ (AM)	-	*Cronobacter sp.*	P		-	-	- (∅)	/	/	/	A	A	
2483242				+ (AM)	-	*Cronobacter sp.*	P		+ 28.14	+ 18.98	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483243				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483244				- (∅)	/	/	A		-	-	- (∅)	/	/	/	A	A	
2483245				+ (AM)	-	*Cronobacter sp.*	P		-	-	- (∅)	/	/	/	A	A	
2483246			1 000 000	+ (AM)	-	*Cronobacter sp.*	P	5/5	+ 25.70	+ 19.71	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	5/5
2483247				+ (AM)	-	*Cronobacter sp.*	P		+ 26.24	+ 18.78	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483248				+ (AM)	-	*Cronobacter sp.*	P		+ 24.08	+ 18.54	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483249				+ (AM)	-	*Cronobacter sp.*	P		+ 27.59	+ 18.66	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	
2483250				+ (AM)	-	*Cronobacter sp.*	P		+ 31.78	+ 18.35	+ (AM)	-	*Cronobacter sp.*	*Cronobacter sp.*	P	P	