



**Method Comparison Study Report
&
Interlaboratory Study Report
for the ISO 16140-2/A1:2024 validation of**

Thermo Scientific™ Cronobacter Precis™ Method

for the detection of *Cronobacter* spp. in infant formula with and without probiotics, infant cereals, ingredients linked to the preparation of infant formula and environmental samples

MicroVal study number: 2020LR93

Method/Kit name: Thermo Scientific™ Cronobacter Precis™ Method

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MicroVal Expert Laboratory: Microsept

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This report contains 45 pages and 59 pages of appendices.

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Foreword

This report is prepared in accordance with ISO 16140-2/A1:2024, MicroVal technical committee interpretation of ISO 16140-2 and MicroVal rules version 9.4 (2024).

Company: Thermo Fisher Scientific

Expert Laboratory: Microsept

Method/Kit name: Thermo Scientific™ Cronobacter Precis™ Method

Validation standard: ISO 16140-2 and its amendment A1 (2024)

Reference method: ISO 22964

Scope of validation:

- infant formula with and without probiotics (10 g)
- environmental samples (25 g or surface samplings)
- infant formula, infant cereals and related ingredients (up to 375 g)

Certification organization: LRQA

List of abbreviations

A(It)	Alternative method
AL	Acceptability Limit
Art. Cont.	artificial contamination
CFU	Colony Forming Units
EL	Expert Laboratory
FP	False Positive
FPR	False Positive Ratio
g	Gram
h	Hour
ILS	Interlaboratory Study
LOD	Level of Detection
MCS	Method Comparison Study
min	minute
ml	millilitre
MR	(MicroVal) Method Reviewer
MVTC	MicroVal Technical Committee
NA	Negative Agreement
na	not applicable
ND	Negative Deviation
neg (-)	negative/no growth/no reaction/target not detected
NS	Non-Suspect growth
nt	not tested
PA	Positive Agreement
PD	Positive Deviation
pos (+)	positive/growth/target detected
PPNA	Presumptive Positive Negative Agreement (belongs to the False Positive results)
PPND	Presumptive Positive Negative Deviation (belongs to the False Positive results)
R(ef)	Reference method
RLOD	Relative Level of Detection
RT	Relative Trueness
S	Suspect growth
SE	Relative Sensitivity
SP	Relative Specificity
TP	True Positive

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Appendices

Initial validation study (2020):

Appendix A: Protocol of the reference method

Appendix B: Protocol of the alternative method

Appendix C: Artificial contaminations

Appendix D: Sensitivity raw results

Appendix E: Relative level of detection raw results

Appendix F: Inclusivity raw results and exclusivity list

Appendix G: Interlaboratory study raw results

Extension study (2023):

Appendix H: Protocol of the reference method

Appendix I: Protocol of the alternative method

Appendix J: Artificial contaminations

Appendix K: Sensitivity raw results

Appendix L: Relative level of detection raw results

1. Introduction

This document introduces the results of the studies for the validation according to the ISO 16140-2/A1:2024 standard of the Thermo Scientific™ Cronobacter Precis™ Method (Microval project n°2020LR93).

For this method, an initial validation was carried out in 2020, and an extension was carried out in 2023.

The initial study (2020) consisted of:

- Interpreting a part of the results obtained during the ISO 16140-2 validation study of the Thermo Scientific™ SureTect™ Cronobacter species PCR Assay, realized by ADRIA Développement, an approved Expert Laboratory at MicroVal,
- Adding validation study data according to the ISO 16140-2 standard from analyses performed in the Expert Laboratory Microsept.

The SureTect Cronobacter species PCR Assay method is validated by AFNOR Certification with the certification number UNI 03/11–12/15 according to the standard ISO 16140-2:2016. This method consists of enriching a test portion in a broth, followed by a lysis step and a detection step using a PCR reaction. Presumptive positive results are confirmed by a direct streaking on Thermo Scientific™ Oxoid™ *Brilliance*™ Chromogenic Cronobacter Isolation Agar (*Brilliance* CCI). Typical colonies are confirmed using the tests described in the ISO 22964 standard.

The results interpreted for the validation of the Cronobacter Precis method are the confirmation results of the SureTect Cronobacter species PCR Assay method, as the workflows are harmonized starting with the same enrichment conditions. This procedure is applied to two categories: Infant formula (10 g) and Environmental samples.

The protocols that were applied to produce the data for the category Infant formula (375 g) are detailed in the following chapters, as well as the exclusivity.

The extension study (2023) consisted of:

- The addition of a new dilution ratio (1-in-6) and of a new incubation time of the enrichment broth for infant formula, infant cereals and related ingredients up to 375 g,
- The addition of a new enrichment protocol for environmental samples.
- Modification of incubation temperature of *Brilliance* CCI agar plates.

The protocols that were applied to produce the data for the category Infant formula (375 g) and environmental samples concerns in this extension are detailed in the following chapters.

2. Method protocols

2.1. Reference method

The method described in the ISO 22964:2017 standard: *Horizontal method for the detection of Cronobacter spp.* will be used as the reference method during this study.

Following the discussions of the Technical Committee during the presentation of the study project, on the proposition of the Expert Laboratory, a test portion of 10 g was used for the reference method, without addition of vancomycin in the initial suspension.

Samples were prepared according to the ISO 6887 standard requirements.

The workflow of the reference method is presented in Appendices A and H.

2.2. Alternative method

2.2.1. Principle of the method

The Cronobacter Precis method consists of an enrichment of a test portion in buffered peptone water (BPW), supplemented with an antibiotic or not (depending on the category), followed by streaking onto a *Brilliance* CCI agar plate.

It is not required to add α -amylase for the analysis of infant cereals as stated in the ISO 6887-5 technical rules.

If isolated typical colonies were observed, the ISO 22964:2017 confirmation procedure was run: Oxidase test together with a biochemical gallery. Oxidase tests may require a purification step on a non-selective agar media before performing because of the color of the typical colonies on chromogenic agars.

It is also possible to use any appropriate ISO 16140-6:2019 validated confirmation method.

2.2.2. Protocols of the method

The Cronobacter Precis method was initially validated for the following protocols and categories:

- **Initial validation protocols:**

The initial validation protocols of the alternative method are presented in Table 1 below.

Table 1: Current protocols of the alternative method per category

Step	Alternative method workflow per step and category				Study design
Enrichment	IF10	Infant formula with and without probiotics	10 g	10 g + 90 ml BPW 16 - 22 h at 34-38°C	Paired data study
	ES	Environmental samples	25 g or surface Sampling	BPW + 6 mg/l vancomycin 18 - 24 h at 34-38°C	Unpaired data study
	IF375	Infant formula, infant cereals and related ingredients	Up to 375 g	375 g + 3375 ml BPW + 6 mg/l vancomycin 20 - 26 h at 34-38°C	Unpaired data study
Isolation	- Streaking of 10 µl on <i>Brilliance</i> CCI agar with a loop Incubation at 41.5 ± 1°C for 24 ± 2 h				
Reading and confirmation	- Typical colonies of <i>Cronobacter</i> appear blue green Confirmations of the typical colonies are performed using the tests described in the reference method with or without purification, a molecular hybridization test or an ISO 16140-6 validated method				

No α-amylase was used for the preparation of initial suspensions of infant cereals.

Oxidase tests may require a purification step on a non-selective agar media before performing because of the color of the typical colonies.

It is possible to store the enrichments for 72 h at 5 ± 3°C before streaking, except for infant formula with probiotics (10 g sample size).

- **Protocols in the extension study:**

This extension study relates to:

- The addition of a new dilution ratio (1-in-6) and of a new incubation time of the enrichment broth for infant formula, infant cereals and related ingredients up to 375 g,
- The addition of a new enrichment protocol for environmental samples.
- A new incubation temperature for *Brilliance* CCI agar plates at 37 ± 1°C.

The protocols of the extension of the alternative method are presented in Table 2 below.

Table 2: Enrichment protocols for the extension study

Category	Type	Test portion size		Enrichment broth	Dilution factor	Enrichment time	Enrichment temperature	Sample preparation
		ISO 22964	Cronobacter Precis					
Infant formula, infant cereals and related ingredients	Infant formula with probiotics	10 g	375 g	Pre-warmed BPW ⁽¹⁾	1-in-6	18 - 24 h	34-38°C	ISO 6887-1, 4, 5
	Infant formula without probiotics	10 g	375 g	Pre-warmed BPW	1-in-6	18 - 24 h	34-38°C	ISO 6887-1, 5
	Ingredients	10 g	375 g	Pre-warmed BPW	1-in-6	18 - 24 h	34-38°C	ISO 6887-1, 5
Environmental samples	Dusts and residues	10 g	25 g	BPW	1-in-10	20 - 26 h	34-38°C	ISO 6887-1, 4, 5
	Cleaning and Process Waters	10 g	25 g	BPW	1-in-10	20 - 26 h	34-38°C	ISO 6887-1
	Surface samples	Surface	Surface	BPW	10 or 100 ml	20 - 26 h	34-38°C	ISO 18593
Isolation	<ul style="list-style-type: none"> - Streaking of 10 µL on 2 <i>Brilliance</i> CCI agar with a loop <li style="padding-left: 20px;"><u>Plate 1</u>: Incubation at 41.5 ± 1°C for 24 ± 2 h <li style="padding-left: 20px;"><u>Plate 2</u>: Incubation at 37 ± 1°C ⁽²⁾ for 24 ± 2 h 							
Reading and confirmation	<ul style="list-style-type: none"> - Typical colonies of <i>Cronobacter</i> appear blue green <p>Confirmations of the typical colonies are performed using the tests described in the reference method with or without purification, a molecular hybridization test or an ISO 16140-6 validated method</p>							

(1) Addition of novobiocin at 6 mg/L for products containing probiotics

(2) A comparison between plates incubated at 41.5 ± 1°C and plates incubated at 37 ± 1°C will be realized.

For samples of infant formula with probiotics up to 10 g the two protocols (with and without novobiocin) can be applied, but beyond a test portion of 10 g and up to 375 g only the protocol with novobiocin can be applied.

The workflow of the method is set out in Appendices B and I.

2.3. Study design

- **Initial validation protocols:**

For the category **IF10**, enrichment broths being common between alternative and reference methods, the study will therefore provide paired data set and the expression “paired study” is used to describe the study design.

No viscosity issues were experienced for infant cereal samples and therefore no α -amylase was added to the reference samples.

For the categories **ES** and **IF375**, either the enrichment broths or the test portion sizes are different, and therefore it is an “unpaired study”.

- **Extension protocols:**

The test samples size tested during this extension, except for the surface samples, was different between the alternative method and the reference method, the design was that of an “unpaired study”.

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 study (2020)

3.1. Methods comparison study

3.1.1. Sensitivity study

Data of the categories **IF10** and **ES** was generated using the data from the validation study of the SureTect Cronobacter species PCR Assay method. As mentioned already, this method includes a confirmation step by streaking of the enriched broth on *Brilliance* CCI agar, followed by confirmation by the biochemical tests described in the reference method.

Artificial contaminations are shown in Appendix C and raw results are shown in Appendix D.

All the data obtained from the isolation steps were used for the interpretation of the sensitivity of the Cronobacter Precis protocols. For the remaining category, **IF375** (that was added to the application scope), the protocols described below were applied.

No particular sample preparation was applied for the alternative method protocol, except for infant formula where the broth was pre-weighed before the addition of the sample.

All samples were homogenized after dilution and before enrichment using a paddle blender.

3.1.1.1. Categories and sample types

Table 3 presents the results per category:

- the data from the SureTect Cronobacter species PCR Assay validation study for categories **IF10** and **ES**,
- the data that were produced during this method comparison study for category **IF375**.

The sensitivity study for all categories concerned 199 samples:

- 133 samples analyzed during the validation study of the SureTect Cronobacter method,
- 66 samples analyzed during the additional study.

Table 3: Distribution of the negative and positive samples per type

Category	Type	Positive	Negative	Total
IF10 Infant formula 10g sample size	a	15	15	30
	b	15	19	34
	Total	30	34	64
ES Environmental samples	a	7	13	20
	b	8	12	20
	c	16	13	29
	Total	31	38	69
IF375 Infant formula, infant cereals and related ingredients 375 g sample size	a	10	12	22
	b	10	13	23
	c	10	11	21
	Total	30	36	66
Total		91	108	199

3.1.1.2. [Test sample preparation](#)

Artificial contamination was carried out using stressed strains in accordance with the requirements of the validation standard and the MicroVal technical rules (see Appendix C).

Table 4 gives the distribution of the positive samples per level of contamination.

Table 4: Distribution of the positive samples per level (cl: contamination level)

Positive samples	Naturally contaminated samples	Artificially contaminated samples						Total
		Spiking			Seeding			
		cl ≤ 5	5 < cl ≤ 10	10 < cl ≤ 30	cl ≤ 3	3 < cl ≤ 10	cl > 10	
91	6	18	0	0	58	9	0	91
/	6.6%	19.8%	0%	0%	63.7%	9.9%	0,0%	100%

There were 91 samples that gave a positive result by at least one of the methods and 6.6% of them were naturally contaminated.

3.1.1.3. [Confirmation protocols](#)

Presumptive positive results were confirmed by the realization of the tests described in the reference method with and without purification, and by biochemical galleries directly on isolated colonies from the *Brilliance* CCI plates.

An additional confirmation protocol was performed, as required by ISO 16140-2:2016, consisting in subculturing 0.1 ml of the enriched BPW + vancomycin broth in 10 ml Thermo Scientific™ Oxoid™ Cronobacter Screening Broth (CSB), incubated for 24 ± 2 h at 41.5 ± 1°C, before streaking 10 µl on *Brilliance* CCI agar, incubated for 24 ± 2 h at 41.5 ± 1°C.

3.1.1.4. [Sensitivity study results](#)

Raw data are shown in Appendix D.

Table 5 shows the results of the sensitivity study for all categories.

Table 5: Results of the sensitivity study for both methods (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)

Category	Response	R+	R-
IF10 Infant formula / 10g sample size	A+	PA = 29	PD = 1
	A-	TND = 0	TNA = 34
ES Environmental samples	A+	PA = 24	PD = 4
	A-	TND = 3	TNA = 38
IF375 Infant formula, infant cereals and related ingredients / 375 g sample size	A+	PA = 19	PD = 7
	A-	TND = 4	TNA = 36
All categories	A+	PA = 72	PD = 12
	A-	TND = 7	TNA = 108

Note: According to the generated data, *C. sakazakii* species shows a higher resistance to desiccation or lyophilization treatments than the other *Cronobacter* species used for artificial contaminations. This observation is confirmed by the naturally contaminated samples of the present extension study where only *C. sakazakii* species were isolated.

3.1.1.5. Sensitivity study calculations

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/A1:2024 standard (Table 6).

Table 6: Calculations of the sensitivities of the reference and alternative methods (SE_{ref} , SE_{alt}), the relative trueness (RT) and the false positive ratio (FPR) per category

Cat.	Type	PA	PA _{FP(alt)}	NA	NA _{FN(alt)}	PD	ND	ND _{FN(alt)}	PD _{FP(alt)}	TND	TNA	SE _{alt}	SE _{ref}	RT	FPR	FNR	
IF10	a	Infant formula without probiotics	15	0	15	0	0	0	0	30	0	0	100%	100%	100%	0%	0.0
	b	Infant formula with probiotics	14	0	19	0	1	0	0	34	0	0	100%	93.3%	97.1%	0%	0.0
	TOTAL		29	0	34	0	1	0	0	64	0	0	100%	96.7%	98.4%	0%	0.0
ES	a	Process and cleaning waters	6	0	13	0	1	0	0	20	0	0	100%	85.7%	95.0%	0%	0.0
	b	Dusts and wastes	6	0	12	0	1	1	0	20	0	0	87.5%	87.5%	90.0%	0%	0.0
	c	Wipe, sponges, wabs	12	0	13	0	2	2	0	29	0	0	87.5%	87.5%	86.2%	0%	0.0
	TOTAL		24	0	38	0	4	3	0	69	0	0	90.3%	87.1%	89.9%	0%	0.0
IF375	a	Infant formula & Infant cereals with probiotics	4	0	12	0	2	4	0	22	0	0	60.0%	80.0%	72.7%	0%	0.0
	b	Infant formula & Infant cereals - probiotics	7	0	13	0	3	0	0	23	0	0	100%	70.0%	87.0%	0%	0.0
	c	Ingredients (caseinates, lecithin, etc...)	8	0	11	0	2	0	0	21	0	0	100%	80.0%	90.5%	0%	0.0
	TOTAL		19	0	36	0	7	4	0	66	0	0	86.7%	76.7%	83.3%	0%	0.0
TOTAL		72	0	108	0	12	7	0	199	0	0	92.3%	86.8%	90.5%	0%	0.0	

The results for all categories are summarized in Table 7 below.

Table 7: Summary of the results for all categories

Parameter	Formula EN ISO 16140-2 :2016	Results for all categories
Sensitivity of the alternative method (SE_{alt})	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \times 100 \%$	92.3 %
Sensitivity of the reference method (SE_{ref})	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \times 100 \%$	86.8 %
Relative trueness (RT)	$RT = \frac{(PA + TNA)}{N} \times 100 \%$	90.5 %
False positive ratio (FPR)	$FPR = \frac{PA_{FP(alt)} + PD_{FP(alt)}}{TNA} \times 100 \%$	0 %
False negative ratio	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	0.0

3.1.1.6. Discordant results

Discordant results are examined according to ISO 16140-2/A1:2024.

The positive deviations are given in Table 8 and the negative deviations in Table 9.

Table 8: Positive deviations

Cat.	#	Item	Cont. level	Reference method		Alternative method
				CCI	Final result	Final result
IF10	2306	Infant formula milk powder with probiotics (7,0.10 ⁵ /g)	4.1	st	A	P
ES	2053	Rinsing water	2.8	st	A	P
	2682	Residues milk powder	1.4	-	A	P
	2058	Wipe milk powder storeroom work plan	1.0	st	A	P
	2440	Wipe pneumatic transport before cleaning	0	-	A	P
IF375	2034828	Infant formula milk powder with probiotics / <i>Bifidobacterium lactis</i> 2,1.10 ⁷ CFU/g	2.8	∅	A	P
	2034845	Infant cereals with probiotics (cocoa) Batch 2 / <i>B. lactis</i> 5,6.10 ⁶ CFU/g	2.2	∅	A	P
	2034851	Infant cereals w/o probiotics (vanilla)	2.0	∅	A	P
	2034888	Infant formula milk powder 6 - 12 months batch 1	1.2	∅	A	P
	2034853	Infant formula milk powder 6 - 12 months batch 2	/	∅	A	P
	2034838	Maltodextrin batch 1	1.8	∅	A	P
	2055441	Rice flour batch 2	/	EM	A	P

Eleven positive deviations were observed with the unpaired study design protocols (ES, IF375) and with artificially (9) and naturally (2) contaminated samples.

In this kind of study design, it is probable that no *Cronobacter* was present in the reference method broth because of the difference of sampling between the two methods.

One positive deviation was observed with the paired study design protocol (IF10). The main hypothesis for this deviation is that the level of *Cronobacter* was low in the initial suspension of the reference method in BPW and that the selection pressure exerted by the vancomycin in the selective enrichment broth (CSB) was too high to reach a detectable concentration of *Cronobacter* on *Brilliance* CCI, whereas a direct streaking on *Brilliance* CCI in the case of the alternative method allowed the detection.

Table 9: Negative deviations

Cat.	#	Item	Cont. level	Reference method	Alternative method		
				Final result	CCI	Final result	ISO conf.
ES	2677	Dusts dairy factory	2.0	P	-	A	st
	2445	Wipe drying tower door after cleaning	1.0	P	-	A	-
	3477	Surface sponge (milk powder factory)	0.2	P	st	A	st
IF375	2034824	Infant formula milk powder with probiotics / <i>Lactobacillus reuteri</i> 5,5.10 ⁶ CFU/g	2.6	P	∅	A	∅
	2034827	Infant formula milk powder with probiotics / <i>Bifidobacterium lactis</i> 4,5.10 ⁶ CFU/g	2.8	P	∅	A	∅
	2034892	Infant cereals with probiotics / <i>B. lactis</i> 6,8 10 ⁶ CFU/g	1.0	P	EL	A	EL
	2066812	Infant cereals with probiotics (chocolate & biscuit) / <i>B. lactis</i> 7.10 ⁵ CFU/g	2.8	P	EL	A	∅

All seven negative deviations were observed with the unpaired study design protocols (ES, IF375) and with artificially contaminated samples. The additional confirmation protocol of the ISO 16140-2 standard did not allow the presence of *Cronobacter* to be detected in the broth of the alternative method.

It is therefore most probable that no *Cronobacter* cells were present in the alternative method broth because of the difference of sampling between the two methods.

Table 10 shows the difference between negative deviations and positive deviations and the acceptability limits.

Table 10: Acceptability limits

Category	Type	ND	PD	Unpaired		Paired				Combined	
				(TND-PD)	AL	(TND-PD)	AL	(TND+PD)	AL	(TND-PD)	AL
IF10	a	0	0	/	/	0	/	0	/	0	/
	b	0	1			-1		1		-1	
	Total	0	1			-1		3		1	
ES	a	0	0	0	/	/	/	/	/	0	/
	b	1	1	0						0	
	c	2	3	-1						-1	
	Total	3	4	-1						3	
IF375	a	4	2	2	/	/	/	/	/	2	/
	b	0	3	-3						-3	
	c	0	2	-2						-2	
	Total	4	7	-3						3	
All categories	Total	7	12	/						-5	5

The observed values (TND – PD) and (TND+PD) are below the acceptability limit for each category and for all categories. The alternative method produces results comparable to the reference method.

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

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

Table 11 shows the evolution of the only result for which the final agreement changed before and after cold storage.

Table 11: Evolution of the results due to the cold storage

Category	Type	N° sample	Before storage	After storage
IF375	b	2034852	NA	PD

Table 12 shows the difference between negative deviations and positive deviations and the acceptability limits after cold storage.

Table 12: Acceptability limits after storage of the broths

Category	Type	ND	PD	Unpaired		Paired				Combined	
				(TND-PD)	AL	(TND-PD)	AL	(TND+PD)	AL	(TND-PD)	AL
IF10	a	0	0	/		0	/	0	/	0	/
	Total	0	0			0	3	0	6	0	
ES	a	0	0	0	/	/				0	
	b	1	1	0						0	
	c	2	3	-1						-1	
	Total	3	4	-1						3	
IF375	a	4	2	2	/	/				2	
	b	0	4	-4						-4	
	c	0	2	-2						-2	
	Total	4	8	-4						3	
All categories	Total	7	12	/				-5	5		

The alternative method produces results comparable to the reference method after storage of the broths for 72 h at $5 \pm 3^\circ\text{C}$.

3.1.1.8. Conclusion of the sensitivity study

The statistical tests of the EN ISO 16140-2/A1:2024 standard conclude that the alternative method produces comparable results to the reference method.

3.1.2. Relative level of detection study

Data of the categories IF10 and ES are generated using the data from the validation study of the SureTect Cronobacter species PCR Assay method. Indeed, this method includes a confirmation procedure by streaking of the enriched broth on Brilliance CCI agar, followed by a confirmation by the tests of the reference method.

The relative level of detection of the category IF375 was determined. The tests and the interpretation of the results were carried out according to the protocols described below.

3.1.2.1. Categories, sample types and strains

Three "food item-strain" pairs were studied in parallel using the reference method and the alternative method, for the studied categories (Table 13).

Table 13: Matrix-strain couple for the RLOD study

Category	Test sample	Matrix	Strain	Inoculation protocol
IF10	10 g	Infant formula without probiotics	<i>Cronobacter sakazakii</i> Ad1418	Lyophilized strain with stabilization after inoculation for 2 weeks at room temperature
	10 g	Infant formula with probiotics		
ES	25	Process water	<i>Cronobacter turicensis</i> Ad1445	Seeding protocol with stabilization for 72 hours at 4°C
IF375	375 g	Infant cereals with probiotics	<i>Cronobacter dublinensis</i> GVV828, wild strain isolated from a milk powder	Lyophilized strain with stabilization after inoculation for 2 weeks at room temperature

The total flora of the matrix was determined and is set out in the results tables in Appendix E.

3.1.2.2. Test sample preparations

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. RLOD study results

The detailed results tables are set out in Appendix E.

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method:
 $RLOD = \frac{LOD_{alt}}{LOD_{ref}}$.

The RLODs calculations were performed according to the standard ISO 16140-2/A1 (2024) 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 14.

The combined RLODs values were calculated according to the weight of the test samples and to the study design.

Table 14: RLODs values for all categories (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, $b=\ln(\text{RLOD})$: logarithm of the RLOD value, $sd(b)$: standard deviation of b , z-Test statistic: absolute value of the test statistic of the z-Test with the null hypothesis $H_0: b=0$, p-value: p-value of the z-Test)

Name	RLOD	RLODL	RLODU	AL
IF10 with probiotics <i>C. sakazakii</i> Ad1418	1.000	0.457	2.187	1,5
IF10 without probiotics <i>C. sakazakii</i> Ad1418	1.000	0.478	2.092	1,5
ES - Process water <i>C. turicensis</i> Ad1445	1.157	0.508	2.635	2,5
IF375 with probiotics <i>C. dublinensis</i> GVV828	1.146	0.498	2.636	2.5
Combined	1.081	0.740	1.578	2.5

The LOD₅₀ calculations according to Wilrich & Wilrich POD-LOD calculation program - version 12, are given in Table 15, valuable data for the method verification by the end-users according to the ISO 16140-3:2020 standard.

Table 15: LOD50% for the alternative and reference method

Matrix/Strain	LOD50% (CFU/per test) Alternative method	LOD50% (CFU/per test) Reference method
IF10 with probiotics / <i>C. sakazakii</i> Ad1418	2.735	2.735
IF10 without probiotics / <i>C. sakazakii</i> Ad1418	1.677	1.677
ES - Process water / <i>C. turicensis</i> Ad1445	0.959	0,813
IF375 with probiotics / <i>C. dublinensis</i> GVV828	1.410	1.254
Combined	1.714	1.623

3.1.2.4. Conclusion RLOD study

The RLODs values are below the acceptability limit set at 2.5 for “un-paired” categories and 1.5 for “paired”, categories meaning that, as stated in ISO 16140-2/A1:2024, 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.

3.1.3. Inclusivity and exclusivity study

With the agreement of the Technical Committee, the inclusivity study data from the SureTect *Cronobacter* species PCR Assay validation study were interpreted.

Concerning the exclusivity study, 30 non-target strains were analyzed.

3.1.3.1. Inclusivity study: protocol and results

A total of 57 *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.

Raw data are given in Appendix F.

All 57 *Cronobacter* spp. isolates gave a positive result with the Cronobacter Precis method.

3.1.3.2. Exclusivity study: protocol and results

The 30 non-target strains were cultured in brain heart infusion broth for 24 h at 37°C, diluted in buffered peptone water in order to obtain levels of around 10^5 cells per ml, then the complete protocol of the method was applied. No vancomycin was added to the enrichment broth to favor the recovery of the non-target strains.

Raw data are given in Appendix F.

No cross-reaction was observed for the non-target strains, except for a strain of *Franconibacter helveticus* which showed blue to blue-green colonies. However, the confirmation tests did not conclude to the presence of *Cronobacter*.

3.1.3.3. Conclusion inclusivity and exclusivity study

The inclusivity and the exclusivity of the alternative method are satisfactory.

3.2. Interlaboratory study

The data of the SureTect *Cronobacter* species PCR Assay validation study from the confirmation step are interpreted according to the ISO 16140-2 standard specifications.

3.2.1. Interlaboratory 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 formulas were first reconstituted in sterile water.

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

- 24 blind coded samples (10 ml) for *Cronobacter* spp. detection by the alternative and reference methods,
- 1 sample (labelled "Sample for anaerobic lactic flora") for anaerobic lactic flora enumeration in de Man Rogosa Sharpe (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 in the reconstituted infant formula were the following:

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

At least one of these inoculated levels should produce fractional positive results.

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 collaborators.

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 48 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 collaborators.

Collaborative study laboratories 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.

The collaborative study instructions were sent on Wednesday 7th October 2015.

3.2.2. Experimental parameters controls

3.2.2.1. Strain and background flora 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 (Table 16).

Table 16: *C. sakazakii* stability in the matrix

Day of analysis	<i>Cronobacter sakazakii</i> 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 *C. 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 (Table 17).

Table 17: 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 upon receipt by the labs and those registered by the thermo-probes, along with the receipt dates are given in Table 18.

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 five Labs (A, F, H, I and J).

Table 18: Sample temperatures at receipt

Laboratories	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	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. Interlaboratory study results

3.2.3.1. Expert laboratory results

The results obtained by the expert lab are given in Table 19. Raw results are in Appendix G.

Table 19: Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	2/8	2/8
L2	8/8	8/8

3.2.3.2. Results obtained by the collaborators

- **Anaerobic lactic flora:**

The enumeration of the anaerobic lactic flora varied from 1.9×10^4 to 1.5×10^5 CFU/g.

- **Cronobacter spp. detection:**

There were 15 collaborators participating in the study. The results obtained are provided in Table 20 (reference method) and Table 21 (alternative method). Raw results are in Appendix G.

Table 20: Positive results by the reference method

Collaborators	Contamination level		
	L0	L1	L2
A	0	7	8
B	0	5	8
C	0	4	8
D	2	6	8
E	0	5	8
F	0	5	8
G	1	4	8
H	0	4	8
I	0	3	8
J	0	5	8
K	0	6	8
L	0	5	8
M	0	5	8
N	0	5	8
O	0	6	8
Total	P₀ = 3	P₁ = 75	P₂ = 120

Two labs found positive results with unspiked samples: Lab D (D22 - D23) and Lab G (G15).

Table 21: Positive results (before and after confirmation) by the alternative method

Collabo- rators	Contamination level								
	L0			L1			L2		
	Brilliance CCI result	Conf.	Final result	Brilliance CCI result	Conf.	Final result	Brilliance CCI result	Conf.	Final result
A	0	0	0	7	7	7	8	8	8
B	0	0	0	5	5	5	8	8	8
C	0	0	0	4	4	4	8	8	8
D	2	2	0	5	5	5	8	8	8
E	0	0	0	5	5	5	8	8	8
F	0	0	0	5	5	5	8	8	8
G	0	0	0	5	5	5	8	8	8
H	0	0	0	4	4	4	8	8	8
I	0	0	0	3	3	3	8	8	8
J	0	0	0	5	5	5	8	8	8
K	0	0	0	6	6	6	8	8	8
L	0	0	0	6	6	6	8	8	8
M	0	0	0	6	6	6	8	8	8
N	0	0	0	4	4	4	8	8	8
O	0	0	0	6	6	6	8	8	8
Total	P₀ = 2	2	CP₀ = 0	P₁ = 76	76	CP₁ = 76	P₂ = 120	120	CP₂ = 120

One lab found positive results with unspiked samples: Lab D (D8 - D23).

Thermo Scientific™ Oxoid™ Microbact™ GNB galleries were provided to run the characteristic colony confirmation. Some labs encountered difficulties with gallery interpretation. Despite the identifications not fitting with the expected results, their results were considered 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 kit.

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

Finally, the Expert Laboratory proposes to remove the labs D and G that are show cross-contaminations in the reference and/or alternative method data. The results from 13 labs are used for interpretation: A, B, C, E, F, H, I, J, K, L, M, N and O.

3.2.3.3. Results of the collaborators retained for interpretation

The results obtained with the 13 labs kept for interpretation are presented in Table 22 (reference method) and Table 23 (alternative method).

Table 22: Positive results by the reference method (Without Lab D and G)

Collaborators	Contamination level		
	L0	L1	L2
A	0	7	8
B	0	5	8
C	0	4	8
E	0	5	8
F	0	5	8
H	0	4	8
I	0	3	8
J	0	5	8
K	0	6	8
L	0	6	8
M	0	6	8
N	0	5	8
O	0	6	8
Total	P₀ = 0	P₁ = 67	P₂ = 104

Table 23: Positive results (before and after confirmation) by the alternative methods (Without Lab D and G)

Collaborators	Contamination level								
	L0			L1			L2		
	Brilliance CCI result	Conf.	Final result	Brilliance CCI result	Conf.	Final result	Brilliance CCI result	Conf.	Final result
A	0	0	0	7	7	7	8	8	8
B	0	0	0	5	5	5	8	8	8
C	0	0	0	4	4	4	8	8	8
E	0	0	0	5	5	5	8	8	8
F	0	0	0	5	5	5	8	8	8
H	0	0	0	4	4	4	8	8	8
I	0	0	0	3	3	3	8	8	8
J	0	0	0	5	5	5	8	8	8
K	0	0	0	6	6	6	8	8	8
L	0	0	0	6	6	6	8	8	8
M	0	0	0	6	6	6	8	8	8
N	0	0	0	4	4	4	8	8	8
O	0	0	0	6	6	6	8	8	8
Total	P₀ = 0	0	CP₀ = 0	P₁ = 66	66	CP₁ = 66	P₂ = 104	104	CP₂ = 104

3.2.4. Calculation and interpretation

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 (Table 24).

Table 24: Percentage of specificity

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

N: number of all L0 tests

P₀ = total number of false-positive results obtained with the blank samples before confirmation

CP₀ = total number of false-positive results obtained with the blank samples

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 (L1).

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 25.

Table 25: 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 = 66	Positive deviation (R-/A+) PD = 0
Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1	Negative agreement (A-/R-) NA = 37

Based on the data summarized in Table 25, 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 (Table 26).

Table 26: Sensitivity, relative trueness and false positive ratio percentages

Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \times 100\% =$	98.5%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \times 100\% =$	100%
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\% =$	99.0%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \times 100\% =$	0.0%

3.2.4.3. Interpretation of 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 14 Labs, the calculated values and the acceptability limits are the following (Table 27).

Table 27: Acceptability limits

	Calculated values	AL	Conclusion
ND - PD	1	4	ND - PD ≤ AL
ND + PD	1	6	ND + PD ≤ AL

The EN ISO 16140-2/A1:2024 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 (Table 28).

Table 28: RLOD - laboratory comparison

Method	LOD50%	LOD95%	RLOD
Reference	0,67 [0,52 ; 0,86]	2,89 [2,25 ; 3,73]	1,03 [0,76-1,38]
Alternative	0,69 [0,53 ; 0,89]	2,97 [2,30 ; 3,83]	

3.3. Conclusion of the initial validation study (2020)

The performance of the Cronobacter Precis method has been assessed according to the ISO 16140-2/A1:2024 standard and the Microval technical rules for the following categories:

- 10 g infant formula,
- environmental samples,
- up to 375 g infant formula with and without probiotics, infant cereals, ingredients linked to the preparation of infant formula.

The alternative method shows satisfying data for all the parts of the ISO 161401-2/A1:2024 study:

- the sensitivity study,
- the relative limit of detection (RLOD) determination,
- the inclusivity and exclusivity testing,
- the interlaboratory study.

The data interpretations are all within the defined acceptability limits and therefore the requirements of the ISO 16140-2/A1:2024 standard.

The Cronobacter Precis method 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 (2023)

4.1. Methods comparison study

4.1.1. Sensitivity study

4.1.1.1. Protocols

- **Incubation time:**

Alternative method agar broth and plates were incubated at the minimal time of the tolerance interval (18 h or 20 h for the broth and 22 h for the plates).

Two *Brilliance* CCI agar plates were streaked. One was incubated at $41.5 \pm 1^\circ\text{C}$ (current protocol) and the other was incubated at $37 \pm 1^\circ\text{C}$. A comparison between the two incubation temperatures was carried out in order to validate the incubation at 37°C .

- **Confirmations:**

Typical colonies of *Cronobacter* (blue green) were confirmed by the tests described in the reference method with or without purification.

A supplementary confirmation protocol was performed from the alternative method samples, as required by the ISO 16140-2:2016 standard, consisting in subculturing 0.1 ml of the enriched BPW or BPW + novobiocin broth into 10 ml CSB, incubated for 24 ± 2 h at $41.5 \pm 1^\circ\text{C}$, before streaking 10 μl onto *Brilliance* CCI agar media, incubated for 24 ± 2 h at $41.5 \pm 1^\circ\text{C}$.

- **Broth storage:**

Storage of the broths for 72 h at $5 \pm 3^\circ\text{C}$ was performed. For positive and discordant samples, enrichment stored broths were streaked on two *Brilliance* CCI agar plate: one was incubated at $41.5 \pm 1^\circ\text{C}$ (current protocol) and the other was incubated at $37 \pm 1^\circ\text{C}$. Typical colonies were confirmed by the tests described in the reference method.

- **Brilliance CCI agar plate storage:**

Storage of the positive and negative *Brilliance* CCI agar plates for 72 h at $5 \pm 3^\circ\text{C}$ will be performed. An interpretation was realized after storage.

- **Interpretation:**

The final results were interpreted in accordance with ISO 16140-2/A1:2024, using the acceptability limits for the unpaired methods according to the categories.

4.1.1.2. Number and nature of the samples

The sensitivity study of the extension concerns 142 samples.

Results are presented in Table 29.

Table 29: Distribution of the negative and positive samples per type

Category	Type	Positive	Negative	Total
Infant formula, infant cereals and related ingredients Test portion: 375 g	a Infant formula & Infant cereals with probiotics	11	10	21
	b Infant formula & Infant cereals without probiotics	9	15	24
	c Ingredients (caseinates, lecithin, etc...)	13	11	24
	Total	33	36	69
Environmental samples	a Dusts and Residues	8	12	20
	b Cleaning and Process Waters	11	15	26
	c Surface samples	12	15	27
	Total	31	42	73
TOTAL (all categories)		64	78	142

4.1.1.3. Artificial contaminations

Artificial contamination was carried out using stressed strains in accordance with the requirements of the validation standard and the MicroVal technical rules (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 tryptone soya yeast extract agar (TSYEA)),
- Seeding protocol with lyophilized strains or air-dried strains stored for 2 weeks at 20°C in the matrix for dehydrated products.
- Seeding protocol for cleaning and process waters.

Table 30 gives the distribution of the positive samples per level of contamination.

Table 30: Distribution of the positive samples per level (cl: contamination level)

Study	Positive samples	Naturally contaminated samples	Artificially contaminated samples						Total
			Spiking			Seeding			
			cl ≤ 5	5 < cl ≤ 10	10 < cl ≤ 30	cl ≤ 3	3 < cl ≤ 10	cl > 10	
Validated	91	6	18	0	0	58	9	0	91
	/	6.6%	19.8%	0%	0%	63.7%	9.9%	0%	100%
Extension	64	0	15	0	0	49	0	0	64
	/	0%	23.4%	0%	0%	76.6%	0%	0%	100%
Total	/	3.9%	21.3%	0%	0%	69.0%	5.8%	0%	100%

In this extension study, 64 samples gave a positive result by at least one of the methods. No naturally contaminated samples were detected. In total, all validations combined, 155 samples gave a positive result by at least one of the methods and 3.9% of them were naturally contaminated.

4.1.1.4. Results

Raw data are shown in Appendix K.

Tables 31 shows the results of the sensitivity study for all categories.

Table 31: 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)

Study	Category	Response	R+	R-
Extension	PIF375	A+	PA = 16	PD = 13
		A-	TND = 4	TNA = 36
	ENV	A+	PA = 22	PD = 3
		A-	TND = 6	TNA = 42
	Total	A+	PA = 38	PD = 16
		A-	TND = 10	TNA = 78

4.1.1.5. Calculation of relative trueness (RT), sensitivity (SE), false positive ratio (FPR) and false negative ratio (FNR)

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 32.

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

<p><u>Sensitivity of the alternative method:</u></p> $SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \times 100 \%$	<p><u>Sensitivity of the reference method:</u></p> $SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \times 100 \%$
<p><u>Relative trueness:</u></p> $RT = \frac{(PA + TNA)}{N} \times 100 \%$	<p><u>False positive ratio:</u></p> $FPR = \frac{PA_{FP(alt)} + PD_{FP(alt)}}{TNA} \times 100 \%$
<p><u>False negative ratio:</u></p> $FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	

Table 32: Values in % of sensitivity for the two methods, relative trueness and false positive ratio for the alternative method (SE_{alt} : sensitivity for the alternative method, SE_{ref} : sensitivity for the reference method, RT: relative trueness, FPR: false positive ratio for the alternative method)

Category	Type	PA	PA _{FP(alt)}	NA	NA _{FN(alt)}	PD	ND	ND _{FN(alt)}	PD _{FP(alt)}	TND	TNA	SE _{alt} %	SE _{ref} %	RT %	FP %	FNR
① Infant formula, infant cereals and related ingredients	a Infant formula with probiotics	8	0	10	0	3	0	0	0	0	10	100,0	72,7	85,7	0,0	0,00
	b Infant formula without probiotics	3	0	15	0	4	2	0	0	2	15	77,8	55,6	75,0	0,0	0,00
	c Ingredients	5	0	11	0	6	2	0	0	2	11	84,6	53,8	66,7	0,0	0,00
	Total	16	0	36	0	13	4	0	0	4	36	87,9	60,6	75,4	0,0	0,00
② Environmental samples	a Dusts and residues	6	0	12	0	1	1	0	0	1	12	87,5	87,5	90,0	0,0	0,00
	b Cleaning and Process Waters	6	0	15	0	2	3	0	0	3	15	72,7	81,8	80,8	0,0	0,00
	c Surface samples	10	0	15	0	0	2	0	0	2	15	83,3	100,0	92,6	0,0	0,00
	Total	22	0	42	0	3	6	0	0	6	42	80,6	90,3	87,7	0,0	0,00
All categories		38	0	78	0	16	10	0	0	10	78	84,4	75,0	81,7	0,0	0,00

The results for the category of the extension study for the two categories tested are summarized in Table 33.

Table 33: Summary of the extension results

Parameter	Formula EN ISO 16140-2/A1 (2024)	Results for all categories
Sensitivity of the alternative method (SE_{alt})	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100 \%$	84.4 %
Sensitivity of the reference method (SE_{ref})	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100 \%$	75.0 %
Relative trueness (RT)	$RT = \frac{(PA + NA)}{N} \times 100 \%$	81.7 %
False positive ratio (FPR)	$FPR = \frac{FP}{NA} \times 100 \%$	0 %
False negative ratio (FNR)	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	0.0

4.1.1.6. Analysis of the discordant results

Discordant results are examined according to the standard ISO 16140-2/A1:2024.

The positive deviations are given in Table 34 and the negative deviations in Table 35.

No difference was seen in the recovery between *Brilliance* CCI agar plates incubated at 37°C and 41.5°C, except for one sample. For simplicity, only results for plates incubated at 41.5°C were included in Tables 34 and 35.

Table 34: Positive deviations

Type	#	Sample	Probiotics	Contamination			Reference method		BPW								
							ISO 22964*		18h at 34-38°C for PIF 20h at 34-38°C for Env.								
				Strain	Conta.	Level CFU	CCI After CSB	Confirmatory tests		Final result	CCI 41,5°C	Confirmation			Final result	Agreement	
			Ox.	Gallery	Ox.	Gallery without purification	Gallery with purification										
a+	2483214	BABYBIO Optima 1	<i>Bifidobacterium lactis</i> 5.1 10 ⁷ CFU/g	<i>C. dublinensis</i>	GVV828	Lyophilisation	1.6	-(Ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
a+	2483216	MODILAC Actigest 2	<i>Bifidobacterium infantis</i> 5.8 10 ⁶ CFU/g	<i>C. sakazakii</i>	HYD216	Lyophilisation	2.0	-(Ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
a+	2428655	Cereals with quinoa, banana, prun	<i>B.lactis</i> 3,4 10 ⁵ CFU/g	<i>C. sakazakii</i>	RLP329	Air-dried	2.0	-(Ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
b+	2428663	Infant cereals brioche - cocoa	/	<i>C. mytjensi</i>	GVV884	Air-dried	2.5	-(EM)	/	/	A	+(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
b+	2498707	Infant cereals cocoa & wheat	/	<i>C. sakazakii</i>	JGG241	Lyophilisation	2.6	-(Ø)	/	/	A	+(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
b+	2498730	Infant formula for 1 at 3 years old batch 1	/	<i>C. dublinensis</i>	GVV828	Lyophilisation	2.4	-(Ø)	/	/	A	+(AL)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
b+	2498731	Infant formula for 1 at 3 years old batch 2	/	<i>C. sakazakii</i>	HXN562	Lyophilisation	2.4	-(Ø)	/	/	A	+(AL)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
c+	2392617	Lactose powder	/	<i>C. sakazakii</i>	HYD216	Lyophilisation	1.2	-(Ø)	/	/	A	+(AL)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
c+	2483276	Skimmed milk powder	/	<i>C. sakazakii</i>	HYD216	Lyophilisation	2.0	-(Ø)	/	/	A	+(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
c+	2392614	Wheat flour	/	<i>C. malonaticus</i>	HSE385	Air-dried	2.4	-(EM)	/	/	A	+(DM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
c+	2392615	Maltodextrin	/	<i>C. sakazakii</i>	JBU888	Air-dried	1.8	-(Ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
c+	2498709	Powdered whole milk	/	<i>C. sakazakii</i>	JGG241	Lyophilisation	1.3	-(Ø)	/	/	A	+(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
c+	2498711	Caseinate	/	<i>C. sakazakii</i>	JGG241	Lyophilisation	2.6	-(EM)	/	/	A	+(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
a+	2483263	Dusts vacuum PIF industry	/	<i>C. dublinensis</i>	DSEL33	Air-dried	3.0	-(Ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
b+	2483198	Process water Ligne 2 R1	/	<i>C. turincensis</i>	HZN090	Seeding	2.0	-(Ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD
b+	2498734	Rinsing water TL+line TL-TR SAEQI	/	<i>C. sakazakii</i>	JFU886	Seeding	3.0	-(EM)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD

Table 35: Negative deviations

Type	#	Sample	Probiotics	Contamination				Reference method		BPW												
								ISO 22964*		18h at 34-38°C for PIF 20h at 34-38°C for Env.												
				Strain	Conta.	Level CFU	CCI After CSB	Ox.	Gallery	Final result	CCI 41,5°C	Ox.	Gallery without purification	Gallery with purification	Final result	Agreement	CCI after CSB	Ox.	Gallery			
b+	2428660	PIF 1er âge batch 2	/	<i>C. sakazakii</i>	HXN562	Lyophilisation	2.2	+	(AM)	-	<i>Cronobacter sp</i>	P	- (ø)	/	/	/	A	ND	- (ø)	/	/	
b+	2498708	Infant formula for 6 at 12 months old	/	<i>C. sakazakii</i>	HXN562	Lyophilisation	2.0	+	(AM)	-	<i>Cronobacter sp</i>	P	- (ø)	/	/	/	A	ND	- (ø)	/	/	
c+	2392616	Whey	/	<i>C. sakazakii</i>	JBU888	Air-dried	1.8	+	(AM)	-	<i>Cronobacter sp</i>	P	-(EM)	/	/	/	A	ND	- (ø)	/	/	
c+	2483272	Soy lecithin	/	<i>C. malonaticus</i>	HSE385	Air-dried	2.4	+	(AM)	-	<i>Cronobacter sp</i>	P	-(EM) Bis:- (EM)	/	/	/	A (FN)	ND	+	(BM)	-	<i>Cronobacter sp</i>
a+	2483264	Dust suction filter weighing powders	/	<i>C. sakazakii</i>	HXA137	Air-dried	2.2	+	(AM)	-	<i>Cronobacter sp</i>	P	- (ø)	/	/	/	A	ND	- (ø)	/	/	
b+	2483199	Dosing water ligne 2 R1	/	<i>C. sakazakii</i>	HXK792	Seeding	1.8	+	(AM)	-	<i>Cronobacter sp</i>	P	- (ø)	/	/	/	A	ND	- (ø)	/	/	
b+	2483200	Rinsing water ligne 1 vide fût	/	<i>C. sakazakii</i>	HZP119	Seeding	1.8	+	(AM)	-	<i>Cronobacter sp</i>	P	- (ø)	/	/	/	A	ND	- (ø)	/	/	
b+	2483203	Rinsing water milk tank 2	/	<i>C. sakazakii</i>	JFU886	Seeding	1.6	+	(AM)	-	<i>Cronobacter sp</i>	P	- (EM)	/	/	/	A	ND	- (EM)	/	/	
c+	2483189	Wipe interior small tank before washing	/	<i>C. turincensis</i>	BTYS37	Spiking	2.2	+	(BM)	-	<i>Cronobacter sp</i>	P	- (EM)	/	/	/	A	ND	- (EM)	/	/	
c+	2483190	Wipe dairy floor	/	<i>C. sakazakii</i>	HXK792	Spiking	4.8	+	(DM)	-	<i>Cronobacter sp</i>	P	- (EM)	/	/	/	A (FN)	ND	+	(BM)	-	<i>Cronobacter sp</i>

Sixteen positive deviations were observed: all from artificially contaminated samples. All positive results obtained by streaking on *Brilliance* CCI agar plates were also confirmed by the additional confirmation protocol of the ISO 16140-2 standard (subculture in CSB before streaking on *Brilliance* CCI).

Ten 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 samples n°2483272 "Soy lecithin" and 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.

In conclusion, the positive deviations and the negative 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.

4.1.1.7. Confirmations

No difference was observed between the confirmation by gallery of identification carried out with or without purification.

For one negative result, n°2483217: "Organic 2nd age infant formula with probiotics", no characteristic colonies could be observed on *Brilliance* CCI agar plate, but there was moderate growth of background microflora on the plates. The additional confirmation protocol of the ISO 16140-2 standard demonstrated the presence of *Cronobacter* in the enrichment broth.

4.1.1.8. Calculation and interpretation of data

Table 36 shows the difference between negative deviations and positive deviations and the acceptability limits.

Table 36: Acceptability limits

Category	Type	N+	TND	PD	Unpaired protocol	
					TND-PD	AL
PIF375	a	11	0	3	/	/
	b	9	2	4		
	c	13	2	6		
	Total	33	4	13	-9	3
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	2	0		
	Total	31	6	3	3	3
All categories		64	10	16	-6	4

The observed values (TND – PD) are below the acceptability limit for each category and for all categories. The alternative method produces results comparable to the reference method.

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

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

Table 37 shows the evolution of the only result for which the final agreement changed before and after cold storage.

Table 37: Evolution of the results due to the cold storage

Category	Type	N° sample	Before storage	After storage
① PIF 375	c	2392617	PD	NA
	c	2392613	PA	ND

Table 38 shows the difference between negative deviations and positive deviations and the acceptability limits after cold storage.

Table 38: Acceptability limits after storage of the broths

Category	Type	N+	TND	PD	Unpaired protocol	
					TND-PD	AL
PIF375	a	11	1	3	/	/
	b	9	2	4		
	c	12	2	5		
	Total	32	5	12	-7	3
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	2	0		
	Total	31	6	3	3	3
All categories		63	11	15	-4	4

The alternative method produces results comparable to the reference method after storage of the broths for 72 h at 5 ± 3°C.

4.1.1.10. Brilliance CCI agar plates storage at 5 ± 3°C for 72 h

All *Brilliance* CCI agar plates (positive and negative) incubated at 41.5°C were stored and then observed again. No difference was observed after cold storage of the plates.

4.1.1.11. Comparison between Brilliance CCI agar plates incubated at 41.5°C versus 37°C

One difference was observed between the *Brilliance* CCI agar plate incubated at 41.5°C versus 37°C for the sample n°2483217 “Infant formula with probiotics”. Table 39 shows the difference between the two incubation temperatures.

Table 39: Comparison of the results between Brilliance CCI agar plate incubated at 41.5°C versus 37°C

Category	Type	N° sample	Brilliance CCI – 41.5°C	Brilliance CCI – 37°C
① PIF 375	a	2483217	NA	PD

Table 40 shows the difference between negative deviations and positive deviations and the acceptability limits for the Brilliance CCI agar plates incubated at 37°C.

Table 40: Acceptability limits for the Brilliance CCI agar plates incubated at 37°C

Category	Type	N+	TND	PD	Unpaired protocol	
					TND-PD	AL
PIF375	a	11	0	4	/	/
	b	9	2	4		
	c	13	2	6		
	Total	33	4	14	-10	3
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	2	0		
	Total	31	6	3	3	3
All categories		64	10	17	-7	-4

The observed values (TND – PD) are below the acceptability limit for each category and for all categories. The alternative method produces results comparable to the reference method with incubation of the Brilliance CCI agar plates at 37°C versus 41.5°C.

After cold storage of the enrichment broths, two Brilliance CCI agar plates were streaked: one incubated at 41.5°C and the other at 37°C. Only one difference was observed between the two incubation temperatures for sample n°2483190 which moved from negative deviation to positive agreement.

Table 41 shows the difference between negative deviations and positive deviations and the acceptability limits for the Brilliance CCI agar plates incubated at 37°C after cold storage of the broths.

Table 41: acceptability limits for the Brilliance CCI agar plates incubated at 37°C after broth storage

Category	Type	N+	TND	PD	Unpaired protocol	
					TND-PD	AL
PIF375	a	11	0	4	/	/
	b	9	2	4		
	c	13	2	6		
	Total	33	4	14	-10	3
ENV	a	8	1	1	/	/
	b	11	3	2		
	c	12	1	0		
	Total	31	5	3	2	3
All categories		64	9	17	-8	-4

All *Brilliance* CCI agar plates (positive and negative) incubated at 37°C were stored and then observed again. No difference was observed after cold storage of the plates.

4.1.1.12. Conclusion of the sensitivity study

The statistical tests of the EN ISO 16140-2/A1:2024 standard conclude that the new protocols of alternative method produces comparable results to the reference method.

4.1.2. Relative level of detection study

4.1.2.1. Matrix-pairs strains

Table 42 summarizes the matrix-strain pairs tested.

Table 42: Matrix-strain pairs for the RLOD study

Category	Test sample	Matrix	Strain	Inoculation protocol
Infant formula, infant cereals and related ingredients	375 g	Infant formula with probiotics	<i>Cronobacter sakazakii</i> JBU888, wild strain isolated from a milk powder	Air-dried strain with stabilization after inoculation for 2 weeks at room temperature
Environmental samples	Surface Sample: stainless steel 1"x1"	Surface sample	<i>Cronobacter turicensis</i> HZN090, wild strain isolated from dairy environment	Seeding protocol

The total flora of the matrix was determined and is set out in the results tables in Appendix L.

4.1.2.2. 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.

The stainless-steel surface used was not sterile and therefore contaminated by the naturally present flora. A count of this flora was carried out on a control sample. For the 1"x1" stainless steel, the low

level was contaminated at a range of 10⁴ CFU/surface area and the high level at a range of 10⁶ CFU/surface area. After drying at room temperature for 16 - 24 h, 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).

4.1.2.3. Results

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

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method: $RLOD = \frac{LOD_{alt}}{LOD_{ref}}$.

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 43.

Table 43: RLODs values for all categories (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, $b = \ln(RLOD)$: logarithm of the RLOD value, $sd(b)$: standard deviation of b , z-Test statistic: absolute value of the test statistic of the z-Test with the null hypothesis $H_0: b=0$, p-value: p-value of the z-Test)

Name	RLOD	RLODL	RLODU
PIF 375 g with probiotics	1.476	0.456	4.778
Environmental sample	1.000	0.339	2.948
Combined	1.160	0.565	2.381

4.1.2.4. Interpretation and conclusion

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

4.1.2.5. Expression of the LOD values

The LOD₅₀ calculations according to Wilrich & Wilrich POD-LOD calculation program - version 12, are given in Table 44.

For the environmental sample category, the spreadsheet used does not allow an LOD_{50%} to be calculated with the contamination protocol used.

Table 44: LOD50% for the alternative and reference method

Categories	Reference method ISO 22964	Alternative method
① PIF375 with probiotics	2.860	4.158

In the context of method verification according to the ISO 16140-3 standard, it is preferable to use Protocol 3 proposed by the standard in order to determine the eLOD₅₀.

4.1.3. Inclusivity and exclusivity study

During initial validation, BPW + vancomycin was used for inclusivity testing. For this extension, an addition of novobiocin is proposed for samples containing probiotics. These two antibiotics have different modes of action: action on DNA gyrase for novobiocin while vancomycin inhibits the synthesis of the peptidoglycan of the bacterial wall. Due to this, the spectrum of action of vancomycin is somewhat broader than that of novobiocin.

Since the protocol applied during the inclusivity study is considered more selective than the protocol proposed in this extension, the Expert laboratory kept the inclusivity and exclusivity data.

4.1.3.1. Inclusivity study: protocol and results

A total of 57 *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.

Raw data are given in Appendix F.

All 57 *Cronobacter* spp. isolates gave a positive result with the Cronobacter Precis method.

4.1.3.2. Exclusivity study: protocol and results

The 30 non-target strains were cultured in brain heart infusion broth for 24 hours at 37°C, diluted in buffered peptone water in order to obtain levels of around 10⁵ cells per ml, then the complete protocol of the method was applied. No vancomycin was added to the enrichment broth to favor the recovery of the non-target strains.

Raw data are given in Appendix F.

No cross-reaction was observed for the non-target strains, except for a strain of *Franconibacter helveticus* which showed blue to blue-green colonies. However, the confirmation tests did not conclude the presence of *Cronobacter*.

4.1.3.3. Conclusion inclusivity and exclusivity study

The inclusivity and the exclusivity of the alternative method are satisfactory.

4.1.4. Conclusion of the extension study

The extension study of the methods was performed according to the EN ISO 16140-2/A1:2024 standard.

- **Sensitivity study**

During this extension, the performance of the Cronobacter Precis method was compared to that of the ISO 22964 reference method by analyzing 142 samples divided into 2 categories.

The observed values (TND – PD) were below the acceptability limits for each category and for all categories after the initial test and after three days of conservation at $5 \pm 3^\circ\text{C}$.

Statistically, the new protocols proposed for the two 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 Cronobacter Precis method and reference method was evaluated by artificially contaminating matrices of the application scope.

The Cronobacter Precis 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).

The extension of the Cronobacter Precis method is considered equivalent to the ISO 22964:2017 method. The performance of the *Brilliance* CCI plate at 37°C was equivalent or better than when incubated at 41.5°C .

5. General conclusion

The data and the interpretation of the method comparison study, interlaboratory study and extension study fulfills the requirements of the standard EN ISO 16140-2/A1:2024. The Cronobacter Precis method for the detection of *Cronobacter* spp. is considered as equivalent to the standard EN ISO 22964:2017.

Le Lion d'Angers, December 17, 2025

Guillaume MESNARD
Method Validation Supervisor



APPENDICES

APPENDIX A

Protocol of the reference method:

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

Infant formula, infant cereals and ingredients:

10 g + 90 mL BPW (ac. ISO 6887 standards requirements)

Environmental samples¹:

1 swab + 10 mL BPW

or 1 sponge + 100 mL BPW or 10 g + 90 mL BPW

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, galleries)

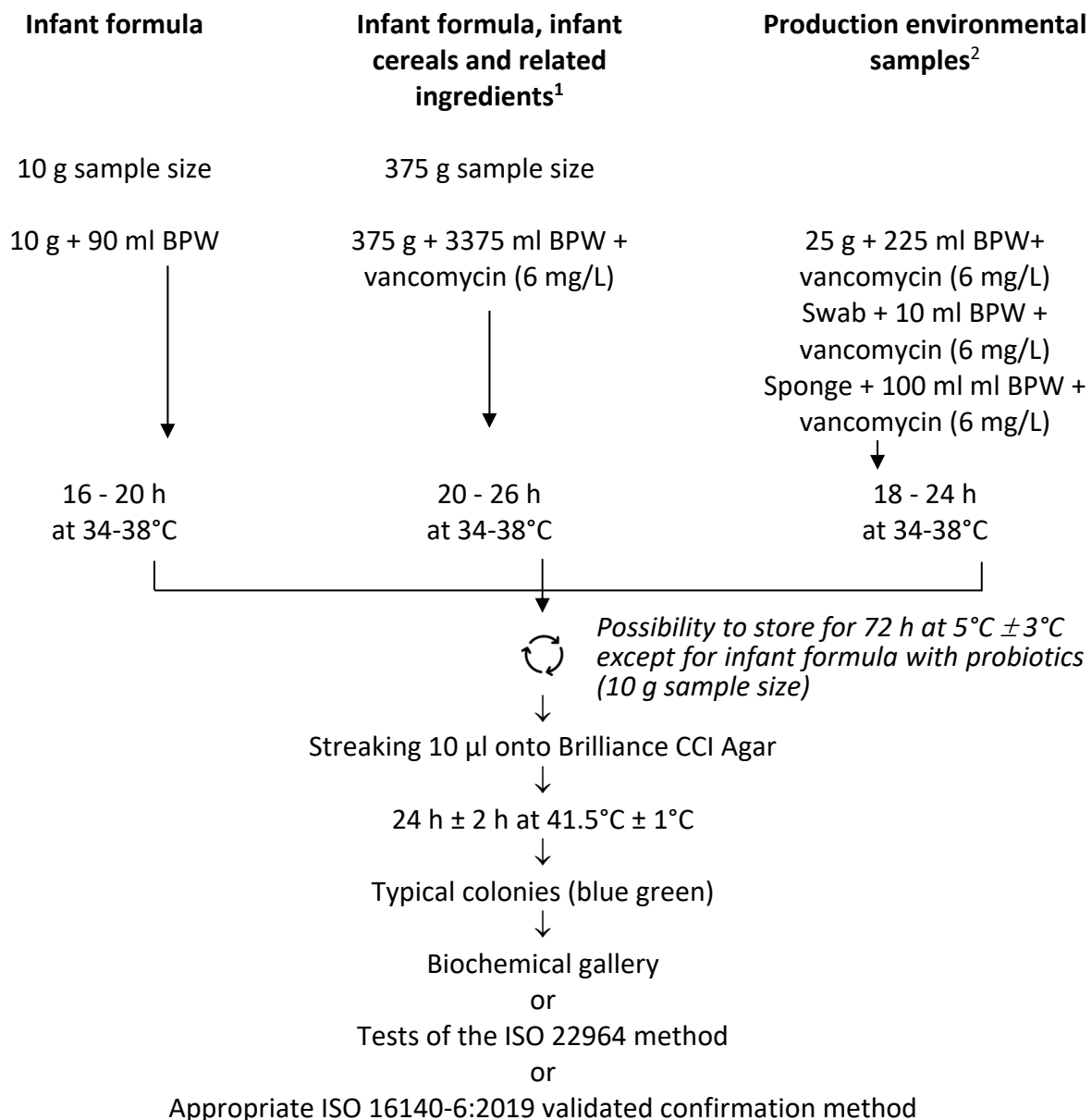
¹ For sampling after cleaning process premoisten

- 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

Protocol of the alternative method:

Cronobacter Precis™



¹ No addition of α-amylase required

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

Sample N°	Product (French name)	Product (probiotics enumeration CFU/g)	Artificial contamination					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measurement	Inoculation level CFU/sample			
2310	Poudre de lait infantile avec probiotiques	Infant formula milk powder with probiotics (3,6.10 ⁶ /g)	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter malonaticus</i> E752	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-1 (0,2)	-	1	a

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4896	Poudre de lait infantile 0-6 mois	Infant formula milk powder	<i>Cronobacter malonaticus</i> E752	Baby food	Spiking-HT 56°C 8 min	>0,90	0-0-0-0-1 (0,2)	-	1	a
4897	Poudre de lait infantile 6-12 mois	Infant formula milk powder	<i>Cronobacter malonaticus</i> 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	<i>Cronobacter dublinensis</i> subsp <i>lactaridi</i> 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	<i>Cronobacter dublinensis</i> subsp <i>lactaridi</i> 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)	<i>Cronobacter dublinensis</i> subsp <i>lactaridi</i> 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)	<i>Cronobacter dublinensis</i> subsp <i>lactaridi</i> 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)	<i>Cronobacter malonaticus</i> 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)	<i>Cronobacter malonaticus</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> Ad 940	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	-	1	a

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5076	Poudre de lait infantile 0-6 mois	Infant formula milk powder	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> Ad 940	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	-	1	a
5078	Poudre de lait infantile 6-12 mois	Infant formula milk powder	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter malonaticus</i> 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)	<i>Cronobacter malonaticus</i> 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)	<i>Cronobacter malonaticus</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> Ad 940	Dairy product	Seeding-Lyophilized strain, 1 week at 20°C	/	1,7	+	1	a

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1455	Poudre de lait infantile avec probiotiques	Infant formula milk powder with probiotics (2,0.10 ⁴ /g)	<i>Cronobacter sakazakii</i> Ad 704	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	0,2	+	1	b
1456	Poudre de lait infantile avec probiotiques	Infant formula milk powder with probiotics (8,0.10 ⁴ /g)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> Ad 1418	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,3	+	1	b

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1466	Poudre de lait infantile avec probiotiques	Infant formula milk powder with probiotics (1,3.10 ⁷ /g)	<i>Cronobacter sakazakii</i> Ad 1418	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	5,3	+	1	b
1467	Poudre de lait infantile avec probiotiques	Infant formula milk powder with probiotics (1,5.10 ⁶ /g)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> 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)	<i>Cronobacter sakazakii</i> Ad 1446	Infant formula milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3,8	+	1	b

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2049	Eau de rinçage	Rinsing water	<i>Cronobacter sakazakii</i> Ad 893	Dairy product	Seeding-48 h 4°C	/	1-3-3-7-5 (3,8)	+	2	a
2050	Eau de rinçage	Rinsing water	<i>Cronobacter sakazakii</i> Ad 947	Dairy product	Seeding-48 h 4°C	/	6-2-3-3-0 (2,8)	+	2	a
2051	Eau de rinçage	Rinsing water	<i>Cronobacter sakazakii</i> Ad 1428	Dairy product	Seeding-48 h 4°C	/	4-3-1-1-1 (2,0)	+	2	a
2052	Eau de rinçage	Rinsing water	<i>Cronobacter sakazakii</i> Ad 1435	Dairy product	Seeding-48 h 4°C	/	3-0-1-5-2 (2,2)	+	2	a
2053	Eau de rinçage	Rinsing water	<i>Cronobacter sakazakii</i> Ad 947	Dairy product	Seeding-48 h 4°C	/	6-2-3-3-0 (2,8)	+	2	a
2446	Eau de process	Process water	<i>Cronobacter sakazakii</i> Ad 1708	Environment 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> Ad 1428	Dairy product	Spiking-pH4 during 7days	0,57	3-0-1-1-0 (1,0)	+	2	b
2677	Poussières laiterie	Dusts	<i>Cronobacter sakazakii</i> Ad 704	Dairy product	Spiking-Lyophilized strain	0,42	2-1-3-0-4 (2,0)	+	2	b
2678	Poussières aspirateur	Dusts	<i>Cronobacter sakazakii</i> Ad 704	Dairy product	Spiking-Lyophilized strain	0,42	2-1-3-0-4 (2,0)	+	2	b
2679	Déchets poudre de lait	Dusts	<i>Cronobacter sakazakii</i> Ad 916	Dairy product	Spiking-Lyophilized strain	0,48	2-3-1-4-4 (2,8)	+	2	b
2680	Poussières laiterie	Dusts	<i>Cronobacter sakazakii</i> Ad 916	Dairy product	Spiking-Lyophilized strain	0,48	2-3-1-4-4 (2,8)	+	2	b
2681	Poussières aspirateur	Dusts	<i>Cronobacter sakazakii</i> Ad 831	Dairy product	Spiking-Lyophilized strain	0,48	0-0-2-3-2 (1,4)	+	2	b
2682	Déchets poudre de lait	Dusts	<i>Cronobacter sakazakii</i> Ad 831	Dairy product	Spiking-Lyophilized strain	0,48	0-0-2-3-2 (1,4)	+	2	b

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3479	Eponge poussières (industrie PDL)	Dusts sponge	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> Ad 940	Milk powder	Spiking-pH4 during 7days	0,56	4-3-3-4-8 (4,4)	+	2	b
2055	Lingette étagère salle de stockage poudre de lait	Wipe	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> 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	<i>Cronobacter sakazakii</i> Ad 1428	Dairy product	Spiking-pH4 during 7days	0,57	3-0-1-1-0 (1,0)	+	2	c
3339	Ecouvillon douchette réserve	Swab	<i>Cronobacter sakazakii</i> Ad 953	Dairy product	Spiking-Lyophilized strain	0,40	0-0-2-2-1 (1,0)	+	2	c

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3340	Ecouvillon dilumate	Swab	<i>Cronobacter sakazakii</i> Ad 1446	Infant formula	Spiking-Lyophilized strain	0,56	2-1-1-3-1 (1,6)	+	2	c
3341	Ecouvillon E5	Swab	<i>Cronobacter sakazakii</i> Ad 953	Dairy product	Spiking-Lyophilized strain	0,40	0-0-2-2-1 (1,0)	-	2	c
3342	Ecouvillon 35	Swab	<i>Cronobacter sakazakii</i> Ad 1446	Infant formula	Spiking-Lyophilized strain	0,56	2-1-1-3-1 (1,6)	+	2	c
3477	Eponge surface (industrie PDL)	Surface sponge	<i>Cronobacter sakazakii</i> Ad 893	Milk powder	Spiking-pH4 during 7days	0,41	0-1-0-0-0 (0,2)	+	2	c
3478	Eponge surface (industrie PDL)	Surface sponge	<i>Cronobacter sakazakii</i> Ad 940	Milk powder	Spiking-pH4 during 7days	0,56	4-3-3-4-8 (4,4)	+	2	c

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		Strain	Code	Origin	Injury protocol	Injury measurement	Inoculation level CFU/sample			
2034825	Infant formula milk powder with probiotics <i>Lactobacillus reuteri</i> 5,5.10 ⁵ CFU/g	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	a
2034826	Infant formula milk powder with probiotics <i>Bifidobacterium infantis</i> 3,1.10 ⁶ CFU/g	<i>Cronobacter malonaticus</i>	HSE835	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.8	-	3	a
2034829	Infant formula milk powder with probiotics <i>Lactobacillus fermentum hereditum</i> 10 ⁶ CFU/g	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	a
2034842	Infant formula milk powder with probiotics <i>Lactobacillus fermentum hereditum</i> 10 ⁶ CFU/g	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.6	-	3	a
2034843	Infant cereals with probiotics (biscuit) <i>B. lactis</i> 7.10 ⁵ CFU/g	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,6	-	3	a
2034844	Infant cereals with probiotics (honey) <i>B. lactis</i> 3,4 10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	JGG241	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.2	-	3	a
2034841	Infant cereals with probiotics <i>B. lactis</i> 6,8 10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	AWX553	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.2	-	3	a
1978475	Infant formula milk powder with probiotics <i>Lactobacillus reuteri</i> 4.10 ⁵ CFU/g	<i>Cronobacter malonaticus</i>	HSE835	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.8	-	3	a
2034846	Infant cereals with probiotics (5 cereals) - <i>B. lactis</i> 5.10 ⁵ CFU/g	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.0	-	3	a
2034847	Infant cereals with probiotics (chocolate & biscuit) - <i>B. lactis</i> 7.10 ⁵ CFU/g	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.4	-	3	a
2034824	Infant formula milk powder with probiotics <i>Lactobacillus reuteri</i> 5,5.10 ⁶ CFU/g	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	+	3	a
2034827	Infant formula milk powder with probiotics <i>Bifidobacterium lactis</i> 4,5.10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	JCT201	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.8	+	3	a
2034828	Infant formula milk powder with probiotics <i>Bifidobacterium lactis</i> 2,1.10 ⁷ CFU/g	<i>Cronobacter sakazakii</i>	JCT201	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.8	+	3	a
2034845	Infant cereals with probiotics (cocoa) Batch 2 - <i>B. lactis</i> 5,6.10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	AWX553	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.2	+	3	a
2034884	Infant formula milk powder with probiotics <i>Lactobacillus reuteri</i> 6,1 10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	JGG241	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.4	+	3	a
2034885	Infant formula milk powder with probiotics <i>Bifidobacterium infantis</i> 3,1.10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	JGG241	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.4	+	3	a
2034886	Infant cereals with probiotics (honey) <i>B. lactis</i> 3,4 10 ⁶ CFU/g	<i>Cronobacter malonaticus</i>	HSE835	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.2	+	3	a

Sample N°	Product	Artificial contamination						Global result	Category	Type
		Strain	Code	Origin	Injury protocol	Injury measurement	Inoculation level CFU/sample			
2034892	Infant cereals with probiotics <i>B. lactis</i> 6,8 10 ⁶ CFU/g	<i>Cronobacter sakazakii</i>	AWX553	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.0	+	3	a
2066812	Infant cereals with probiotics (chocolate & biscuit) - <i>B. lactis</i> 7.10 ⁵ CFU/g	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Air-dried strain, 1 week at 20°C	/	2.8	+	3	a
2034830	Infant formula milk powder 6 - 12 months	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	b
2034831	Infant formula milk powder 0 - 6 months	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	b
2034832	Infant formula milk powder 12 - 36 months batch 1	<i>Cronobacter malonaticus</i>	HSE835	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.8	-	3	b
2034833	Infant formula milk powder 12 - 36 months batch 2	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.6	-	3	b
2034834	Infant cereals w/o probiotics	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.6	-	3	b
2034835	Infant formula milk powder 0 - 6 months	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	b
2034848	Infant formula milk powder 12 - 36 months batch 2	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2,6	-	3	b
2034849	Infant cereals w/o probiotics (biscuit)	<i>Cronobacter sakazakii</i>	JGG241	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.2	-	3	b
2034850	Infant cereals w/o probiotics (wheat & vanilla)	<i>Cronobacter sakazakii</i>	JGG241	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.2	-	3	b
2034852	Infant cereals w/o probiotics (wheat & cocoa)	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.4	-	3	b
2066815	Infant formula milk powder 0 - 6 months	<i>Cronobacter sakazakii</i>	HYD216	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.0	+	3	b
1978477	Infant cereals w/o probiotics	<i>Cronobacter sakazakii</i>	AWX553	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.2	+	3	b
2034851	Infant cereals w/o probiotics (vanilla)	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.0	+	3	b
2034888	Infant formula milk powder 6 - 12 months batch 1	<i>Cronobacter malonaticus</i>	HSE835	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.2	+	3	b
2034889	Infant formula milk powder 0 - 6 months	<i>Cronobacter sakazakii</i>	HXN562	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3.0	+	3	b

Sample N°	Product	Artificial contamination						Global result	Category	Type
		Strain	Code	Origin	Injury protocol	Injury measurement	Inoculation level CFU/sample			
2034890	Infant formula milk powder 12 - 36 months batch 1	<i>Cronobacter sakazakii</i>	HXN562	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3.0	+	3	b
2034891	Infant formula milk powder 12 - 36 months batch 2	<i>Cronobacter sakazakii</i>	JCT201	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1	+	3	b
2066813	Infant cereals w/o probiotics (wheat & cocoa)	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	3.0	+	3	b
2066814	Infant cereals w/o probiotics (biscuit)	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 1 week at 20°C	/	3.2	+	3	b
2034836	Caseinate powder	<i>Cronobacter sakazakii</i>	HXN562	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	1.2	-	3	c
2034837	Lactoserum powder	<i>Cronobacter turicensis</i>	HZN090	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	c
2034839	Lecithine soja powder	<i>Cronobacter sakazakii</i>	AWX553	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.2	-	3	c
2034840	Lactose powder	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	-	3	c
1978476	Lactoserum permeat batch 2	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.4	-	3	c
1978478	Lactoserum permeat batch 1	<i>Cronobacter sakazakii</i>	JGG241	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.2	-	3	c
2034838	Maltodextrine batch 1	<i>Cronobacter malonaticus</i>	HSE835	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	1.8	+	3	c
1978479	Rice flour batch 1	<i>Cronobacter sakazakii</i>	AWX553	Cereal	Seeding-Air-dried strain, 2 weeks at 20°C	/	2.2	+	3	c
1978480	Powdered whole milk	<i>Cronobacter dublinensis</i>	GVV828	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	2.6	+	3	c
2034887	Lactoserum powder	<i>Cronobacter sakazakii</i>	HXN562	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3.0	+	3	c
2034893	Lactose	<i>Cronobacter sakazakii</i>	HXN562	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3.0	+	3	c
2034894	Lecithine soja powder	<i>Cronobacter sakazakii</i>	HXN562	Environment milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	3.0	+	3	c
2034895	Powdered whole milk	<i>Cronobacter sakazakii</i>	JCT201	Milk powder	Seeding-Lyophilized strain, 2 weeks at 20°C	/	<1	+	3	c

Sample N°	Product	Artificial contamination						Global result	Category	Type
		Strain	Code	Origin	Injury protocol	Injury measurement	Inoculation level CFU/sample			
2066816	Lactoserum permeat	<i>Cronobacter muytjensii</i>	GVW884	Collection	Seeding-Air-dried strain, 1 week at 20°C	/	3.2	+	3	c
2066817	Wheat flour	<i>Cronobacter sakazakii</i>	HYD216	Environment milk powder	Seeding-Lyophilized strain, 1 week at 20°C	/	2.0	+	3	c

APPENDIX D

Sensitivity raw results

Key:

- A : *Cronobacter* not detected
- P : *Cronobacter* detected
- PA : positive agreement
- NA : negative agreement
- PD : positive deviation
- ND : negative deviation

Category		Types	
IF10	Infant formula milk powders 10g	a	Infant formula milk powder without probiotics
		b	Infant formula milk powder with probiotics
ES	Environmental samples	a	Process and cleaning waters
		b	Dusts and wastes
		c	Wipes, sponges, swabs

- + : positive result / presence of typical colonies
- : negative result / absence of typical colonies
- / : test not realized
- st : absence of growth of any colony on the Petri dish
- (x) : number of typical colonies

Category		Types	
IF375	Infant formula milk powder 375g	a	Infant formula milk powder with probiotics
		b	Infant formula milk powder without probiotics
		c	Ingredients

Bacterial burden

∅: no culture

L = low

M = moderate

H = high

Breakdown 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 *Listeria* if $x \leq 5$

Infant formula - 10 g

Sample N°	Product (probiotics enumeration CFU/g)	Type	Global result	ISO 22964				Cronobacter Precis									
				CCI	Confirmation tests		Final result	16 h at 37°C -BPW					Enrichment broth storage 72h 2-8°C				
					OX	Gallery		CCI	Confirmation		Final result	Add. conf. CCI (streaking after subculture in CSB)	Agreement	CCI	Final result	Add. conf. CCI (streaking after subculture in CSB)	Agreement
									ox	Gallery							
4885	Infant formula milk powder 6-12 months	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4886	Infant formula milk powder 0-6 months	a-	-	st	/	/	A	-	/	/	A	st	NA	/	/	/	/
4887	Infant formula milk powder 0-6 months	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4888	Infant formula milk powder 0-36 months	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4890	Infant formula milk powder 6-12 months	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4895	Infant formula milk powder growth	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4896	Infant formula milk powder 0-6 months	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4897	Infant formula milk powder 6-12 months	a-	-	st	/	/	A	-	/	/	A	st	NA	/	/	/	/
4898	Infant formula milk powder 0-6 months	a-	-	st	/	/	A	-	/	/	A	st	NA	/	/	/	/
4899	Infant formula milk powder 6-12 months	a-	-	st	/	/	A	-	/	/	A	st	NA	/	/	/	/
5075	Infant formula milk powder 6-12 months	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
5077	Infant formula milk powder 0-6 months	a-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA
5203	Organic infant formula milk powder	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
5204	Infant formula milk powder - growth	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
5205	Infant formula milk powder - growth	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4889	Infant formula milk powder growth	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
4891	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
4892	Infant formula milk powder 6-12 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
4893	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
4894	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5070	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5071	Infant formula milk powder 6-12 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5072	Infant formula milk powder 1-3 years	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5073	Infant formula milk powder 6-12 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5074	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5076	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5078	Infant formula milk powder 6-12 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5079	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5080	Infant formula milk powder 0-6 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5081	Infant formula milk powder 6-12 months	a+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1457	Infant formula milk powder with probiotics (1,6.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1459	Infant formula milk powder with probiotics (5,8.10 ⁷ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1462	Infant formula milk powder with probiotics (<2,0.10 ⁷ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1463	Infant formula milk powder with probiotics (<2,0.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1464	Infant formula milk powder with probiotics (<2,0.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1467	Infant formula milk powder with probiotics (1,5.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1468	Infant formula milk powder with probiotics (4,0.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1469	Infant formula milk powder with probiotics (1,2.10 ⁷ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
2303	Infant formula milk powder with probiotics (4,7.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
2305	Infant formula milk powder with probiotics (6,0.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
2310	Infant formula milk powder with probiotics (3,6.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4900	Infant formula milk powder with probiotics (4,0.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4901	Infant formula milk powder with probiotics (1,8.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4902	Infant formula milk powder with probiotics (<200/g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4903	Infant formula milk powder with probiotics (4,0.10 ⁶ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/

Infant formula - 10 g

Sample N°	Product (probiotics enumeration CFU/g)	Type	Global result	ISO 22964				Cronobacter Precis									
				CCI	Confirmation tests		Final result	16 h at 37°C -BPW					Enrichment broth storage 72h 2-8°C				
					OX	Gallery		CCI	Confirmation		Final result	Add. conf. CCI (streaking after subculture in CSB)	Agreement	CCI	Final result	Add. conf. CCI (streaking after subculture in CSB)	Agreement
									ox	Gallery							
5082	Infant formula milk powder with probiotics (<200/g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
5206	Infant formula milk powder with probiotics (1,4.10 ³ /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
5207	Infant formula milk powder with probiotics (2,0.10 ² /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
5208	Infant formula milk powder with probiotics (4,0.10 ² /g)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
1455	Infant formula milk powder with probiotics (2,0.10 ⁴ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1456	Infant formula milk powder with probiotics (8,0.10 ⁴ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1458	Infant formula milk powder with probiotics (1,0.10 ⁵ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1460	Infant formula milk powder with probiotics (<2,0.10 ⁵ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1461	Infant formula milk powder with probiotics (<2,0.10 ⁵ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1465	Infant formula milk powder with probiotics (2,2.10 ⁶ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
1466	Infant formula milk powder with probiotics (1,3.10 ⁷ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2304	Infant formula milk powder with probiotics (5,4.10 ⁶ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2306	Infant formula milk powder with probiotics (7,0.10 ⁵ /g)	b+	+	st	/	/	A	+	-	+	P	st	PD	+	P	+	PD
2307	Infant formula milk powder with probiotics (8,0.10 ⁵ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2308	Infant formula milk powder with probiotics (3,2.10 ⁵ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2309	Infant formula milk powder with probiotics (8,8.10 ⁵ /g)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5240	Infant formula milk powder with probiotics (7,9.10 ⁶ /g)	b+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5241	Infant formula milk powder with probiotics (4,0.10 ⁴ /g)	b+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA
5242	Infant formula milk powder with probiotics (2,0.10 ⁴ /g)	b+	+	+p	-	+	P	+	-	+	P	+	PA	+	P	+	PA

Environmental samples

Sample N°	Product	Type	Global result	ISO 22964				Cronobacter Precis									
				CCI	Confirmation tests		Final result	18h at 37°C -BPW +Vancomycin (6mg/L)					Enrichment broth storage 72h 2-8°C				
					OX	Gallery		CCI	Confirmation		Final result	Add. conf. CCI (streaking after subculture in CSB)	Agreement	CCI	Final result	Add. conf.	
									ox	Gallery						CCI (streaking after subculture in CSB)	Agreement
2446	Process water	a-	-	-	/	/	A	-	/	/	A	-	NA	-	A	-	NA
2447	Cleaning water	a-	-	-	/	/	A	-	/	/	A	-	NA	/	/	/	/
4075	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4076	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA
4077	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4078	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4079	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4085	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA
4086	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA
4087	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA
4024	Process water (pastry)	a-	-	-	/	/	A	-	/	/	A	-	NA	/	/	/	/
4027	Rinsing water	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4028	Process water	a-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
2049	Rinsing water	a+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2050	Rinsing water	a+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2051	Rinsing water	a+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2052	Rinsing water	a+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2053	Rinsing water	a+	+	st	/	/	A	+	-	+	P	+	PD	+	P	+	PD
4398	Rinsing water (milk powder factory)	a+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
4399	Rinsing water	a+	+	+	-	+	P	+	-	+	P	+	PA	+(1)	P	+	PA
3479	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	-	/	/	A	-	NA	/	/	/	/
3480	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	-	/	/	A	st	NA	/	/	/	/
3488	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	st	/	/	A	st	NA	/	/	/	/
3489	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	st	/	/	A	st	NA	/	/	/	/
3490	Dusts sponge (milk powder factory)	b-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA
3491	Dusts sponge (milk powder factory)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
3492	Dusts sponge (milk powder factory)	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
3493	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	st	/	/	A	st	NA	/	/	/	/
3494	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	-	/	/	A	-	NA	/	/	/	/
3495	Dusts sponge (milk powder factory)	b-	-	-	/	/	A	-	/	/	A	-	NA	/	/	/	/
4025	Wipe milk powder factory dusts	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
4026	Wipe dusts	b-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/
2448	Siphon water	b+	+	+	-	+	P	+d/+	-	+	P	+	PA	+	P	+	PA
2677	Dusts dairy factory	b+	+	+	-	+	P	-	/	/	A	st	ND	-	A	-	ND
2678	Dusts industrial vacuum cleaner	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2679	Residues milk powder	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2680	Dusts dairy factory	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2681	Dusts industrial vacuum cleaner	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
2682	Residues milk powder	b+	+	-	/	/	A	+	-	+	P	+	PD	+	P	+	PD
3481	Dusts sponge (milk powder factory)	b+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA
3343	Swab	c-	-	-	/	/	A	-	/	/	A	-	NA	/	/	/	/
3344	Swab	c-	-	st	/	/	A	st	/	/	A	-	NA	/	/	/	/

Environmental samples

Sample N°	Product	Type	Global result	ISO 22964				Cronobacter Precis										
				CCI	Confirmation tests		Final result	18h at 37°C -BPW +Vancomycin (6mg/L)					Enrichment broth storage 72h 2-8°C					
					OX	Gallery		CCI	Confirmation		Final result	Add. conf. CCI (streaking after subculture in CSB)	Agreement	CCI	Final result	Add. conf.		
									ox	Gallery						CCI (streaking after subculture in CSB)	Agreement	
3345	Swab	c-	-	st	/	/	A	st	/	/	A	-	NA	/	/	/	/	
3341	Swab	c-	-	st	/	/	A	st	/	/	A	-	NA	/	/	/	/	
3482	Surface sponge (milk powder factory)	c-	-	st	/	/	A	-	/	/	A	st	NA	-	A	-	NA	
3485	Surface sponge (milk powder factory)	c-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA	
3486	Surface sponge (milk powder factory)	c-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/	
3487	Surface sponge (milk powder factory)	c-	-	-	/	/	A	st	/	/	A	st	NA	-	A	st	NA	
4080	Swab ground holder	c-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/	
4081	Swab ground walkway	c-	-	st	/	/	A	st	/	/	A	st	NA	st	A	st	NA	
4082	Swab ground circulation	c-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/	
4083	Swab mixer	c-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/	
4084	Swab	c-	-	st	/	/	A	st	/	/	A	st	NA	/	/	/	/	
2055	Wipe milk powder storeroom shelf	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
2056	Wipe milk powder storeroom shelf	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
2057	Wipe milk powder storeroom work plan	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
2058	Wipe milk powder storeroom work plan	c+	+	st	/	/	A	+	-	+	P	+	PD	+	P	+	PD	
2440	Wipe pneumatic transport before cleaning	c+	+	-	/	/	A	+d/+	-	+	P	+	PD	+	P	+	PD	
2441	Wipe ground	c+	+	+	-	+	P	+d/+	-	+	P	+	PA	+	P	+	PA	
2443	Wipe pneumatic transport after cleaning	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
2444	Wipe ground after cleaning	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
2445	Wipe drying tower door after cleaning	c+	+	+	-	+	P	-	/	/	A	-	ND	-	A	-	ND	
3339	Swab stock barcode scanner	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
3340	Swab dilutor	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
3342	Swab	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
3477	Surface sponge (milk powder factory)	c+	+	+	-	+	P	st	/	/	A	st	ND	st	A	st	ND	
3478	Surface sponge (milk powder factory)	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
3483	Surface sponge (milk powder factory)	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	
3484	Surface sponge (milk powder factory)	c+	+	+	-	+	P	+	-	+	P	+	PA	+	P	+	PA	

Infant formula 375 g

Sample N°	Type	Product (probiotics enumeration CFU/g)	Inoculation Level	Reference method				Cronobacter Precis method												
				ISO 22964 (ø)				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	CCI	Confirmation tests		Final result	ISO confirmation tests			CCI	Confirmation tests		Final result		
					Ox	Gallery			Ox	Gallery		CCI After CSB	Ox			Gallery	Ox			Gallery
2055439	a-	Infant cereals with probiotics (milk chocolate) / <i>B.lactis</i> 6,8.10 ⁶ CFU/g	/	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034825	a-	Infant formula milk powder with probiotics / <i>Lactobacillus reuteri</i> 5,5.10 ⁵ CFU/g	2,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034826	a-	Infant formula milk powder with probiotics / <i>Bifidobacterium infantis</i> 3,1.10 ⁶ CFU/g	1,8	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034829	a-	Infant formula milk powder with probiotics / <i>Lactobacillus fermentum hereditum</i> 10 ⁶ CFU/g	2,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034842	a-	Infant formula milk powder with probiotics / <i>Lactobacillus fermentum hereditum</i> 10 ⁵ CFU/g	1,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034843	a-	Infant cereals with probiotics (biscuit) / <i>B. lactis</i> 7.10 ⁷ CFU/g	2,6	Ø	/	/	A	EM	/	/	A	EM	/	/	NA	EM	/	/	A	NA
2034844	a-	Infant cereals with probiotics (honey) / <i>B. lactis</i> 3,4 10 ⁵ CFU/g	2,2	Ø	/	/	A	EL	/	/	A	EL	/	/	NA	EL	/	/	A	NA
2034841	a-	Infant cereals with probiotics / <i>B. lactis</i> 6,8 10 ⁶ CFU/g	2,2	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
1978475	a-	Infant formula milk powder with probiotics / <i>Lactobacillus reuteri</i> 4.10 ⁵ CFU/g	1,8	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034846	a-	Infant cereals with probiotics (5 cereals) / <i>B. lactis</i> 5.10 ⁵ CFU/g	2,0	Ø	/	/	A	DL (1 colony ⁻) CCI bis: -	-	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034847	a-	Infant cereals with probiotics (chocolate & biscuit) / <i>B. lactis</i> 7.10 ⁵ CFU/g	2,4	Ø	/	/	A	DL (1 colony ⁻) CCI bis: -	-	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2045398	a-	Infant cereals with probiotics (5 cereals) / <i>B. lactis</i> 5.10 ⁵ CFU/g	/	Ø	/	/	A	EL	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034824	a+	Infant formula milk powder with probiotics / <i>Lactobacillus reuteri</i> 5,5.10 ⁵ CFU/g	2,6	AM	-	Cronobacter	P	Ø	/	/	A	Ø	/	/	ND	EL	/	/	A	ND
2034827	a+	Infant formula milk powder with probiotics / <i>Bifidobacterium lactis</i> 4,5.10 ⁶ CFU/g	2,8	AM	-	Cronobacter	P	Ø	/	/	A	Ø	/	/	ND	Ø	/	/	A	ND
2034828	a+	Infant formula milk powder with probiotics / <i>Bifidobacterium lactis</i> 2,1.10 ⁷ CFU/g	2,8	Ø	/	/	A	AM	-	Cronobacter	P	AM	-	Cronobacter	PD	AM	-	Cronobacter	P	PD
2034845	a+	Infant cereals with probiotics (cocoa) Batch 2 / <i>B. lactis</i> 5,6.10 ⁶ CFU/g	2,2	Ø	/	/	A	AM	-	Cronobacter	P	AM	-	Cronobacter	PD	AM	-	Cronobacter	P	PD
2034884	a+	Infant formula milk powder with probiotics / <i>Lactobacillus reuteri</i> 6,1 10 ⁵ CFU/g	2,4	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AL	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2034885	a+	Infant formula milk powder with probiotics / <i>Bifidobacterium infantis</i> 3,1.10 ⁵ CFU/g	2,4	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2034886	a+	Infant cereals with probiotics (honey) / <i>B. lactis</i> 3,4 10 ⁵ CFU/g	1,2	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2034892	a+	Infant cereals with probiotics / <i>B. lactis</i> 6,8 10 ⁶ CFU/g	1,0	AL	-	Cronobacter	P	EL	/	/	A	EL	/	/	ND	EL	/	/	A	ND
2045399	a+	Infant cereals with probiotics (cocoa) Batch 1 / <i>B. lactis</i> 5,6.10 ⁵ CFU/g	/	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2066812	a+	Infant cereals with probiotics (chocolate & biscuit) / <i>B. lactis</i> 7.10 ⁵ CFU/g	2,8	AM	-	Cronobacter	P	EL	/	/	A	Ø	/	/	ND	EL	/	/	A	ND
2034830	b-	Infant formula milk powder 6 - 12 months	2,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034831	b-	Infant formula milk powder 0 - 6 months	2,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034832	b-	Infant formula milk powder 12 - 36 months batch 1	1,8	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034833	b-	Infant formula milk powder 12 - 36 months batch 2	1,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034834	b-	Infant cereals w/o probiotics	1,6	Ø	/	/	A	CM blue & white colonies BIS: CCI -/-/-/-	-	ID32E: Ø identification <i>Acinetobacter baumanii</i>	A	EM white colonies	/	/	NA	EM white colonies	/	/	A	NA
2034835	b-	Infant formula milk powder 0 - 6 months	2,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034848	b-	Infant formula milk powder 12 - 36 months batch 2	2,6	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2034849	b-	Infant cereals w/o probiotics (biscuit)	2,2	Ø	/	/	A	EL	/	/	A	Ø	/	/	NA	EL	/	/	A	NA
2034850	b-	Infant cereals w/o probiotics (wheat & vanilla)	2,2	EL	/	/	A	EL	/	/	A	Ø	/	/	NA	EL	/	/	A	NA
2034852	b-	Infant cereals w/o probiotics (wheat & cocoa)	2,4	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	AM	-	Cronobacter	P	PD
2045400	b-	Infant formula milk powder w/o probiotics 0-6 months	/	Ø	/	/	A	EM	/	/	A	EL	/	/	NA	EL	/	/	A	NA
2045401	b-	Infant formula milk powder w/o probiotics 6-12 months	/	Ø	/	/	A	EM	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2045403	b-	Infant formula milk powder w/o probiotics 6-12 months	/	Ø	/	/	A	Ø	/	/	A	Ø	/	/	NA	Ø	/	/	A	NA
2066815	b+	Infant formula milk powder 0 - 6 months	2,0	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
1978477	b+	Infant cereals w/o probiotics	2,2	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2034851	b+	Infant cereals w/o probiotics (vanilla)	2,0	Ø	/	/	A	AM	-	Cronobacter	P	AL	-	Cronobacter	PD	AM	-	Cronobacter	P	PD
2034888	b+	Infant formula milk powder 6 - 12 months batch 1	1,2	Ø	/	/	A	AL	-	Cronobacter	P	AL	-	Cronobacter	PD	AL	-	Cronobacter	P	PD
2034889	b+	Infant formula milk powder 0 - 6 months	3,0	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2034890	b+	Infant formula milk powder 12 - 36 months batch 1	3,0	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2034891	b+	Infant formula milk powder 12 - 36 months batch 2	<1	AL	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AL	-	Cronobacter	P	PA
2034853	b+	Infant formula milk powder 6 - 12 months batch 2	/	Ø	/	/	A	AM	-	Cronobacter	P	AL	-	Cronobacter	PD	AL	-	Cronobacter	P	PD
2066813	b+	Infant cereals w/o probiotics (wheat & cocoa)	3,0	AL	-	Cronobacter	P	AM	-	Cronobacter	P	AL	-	Cronobacter	PA	AM	-	Cronobacter	P	PA
2066814	b+	Infant cereals w/o probiotics (biscuit)	3,2	AL	-	Cronobacter	P	AL	-	Cronobacter	P	AL	-	Cronobacter	PA	AM	-	Cronobacter	P	PA

Infant formula 375 g

Sample N°	Type	Product (probiotics enumeration CFU/g)	Inoculation Level	Reference method				Cronobacter Precis method													
				ISO 22964 (0)				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	CCI	Confirmation tests		Final result	ISO confirmation tests				CCI	Confirmation tests		Final result		
					Ox	Gallery			Ox	Gallery		CCI After CSB	Ox	Gallery			Ox	Gallery			
2034836	c-	Caseinate powder	1,2	∅	/	/	A	∅	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
2034837	c-	Whey powder	2,6	∅	/	/	A	∅	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
2034839	c-	Lecithin soy powder	2,2	∅	/	/	A	EL	/	/	A	EL	/	/	NA	∅	/	/	A	NA	
2034840	c-	Lactose powder	2,6	∅	/	/	A	∅	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
1978476	c-	Whey permeate batch 2	2,4	∅	/	/	A	EM	/	/	A	EM white colonies	/	/	NA	EM white colonies	/	/	A	NA	
1978478	c-	Whey permeate batch 1	2,2	∅	/	/	A	∅	/	/	A	EL white colonies	/	/	NA	EL white colonies	/	/	A	NA	
2045402	c-	Maltodextrin	/	∅	/	/	A	EM	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
2055440	c-	Honey powder	/	∅	/	/	A	∅	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
2055442	c-	Powdered whole milk	/	∅	/	/	A	∅	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
2055443	c-	Wheat flour	/	∅	/	/	A	∅	/	/	A	∅	/	/	NA	EL	/	/	A	NA	
2055444	c-	Wheat starch	/	∅	/	/	A	∅	/	/	A	∅	/	/	NA	∅	/	/	A	NA	
2034838	c+	Maltodextrin batch 1	1,8	∅	/	/	A	AM	-	Cronobacter	P	AM	-	Cronobacter	PD	AM	-	Cronobacter	P	PD	
1978479	c+	Rice flour batch 1	2,2	AL	-	Cronobacter	P	AM	-	Cronobacter	P	BM	-	Cronobacter	PA	BM	-	Cronobacter	P	PA	
1978480	c+	Powdered whole milk	2,6	AM	-	Cronobacter	P	AH	-	Cronobacter	P	AH	-	Cronobacter	PA	AH	-	Cronobacter	P	PA	
2034887	c+	Whey powder	3,0	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AL	-	Cronobacter	PA	AM	-	Cronobacter	P	PA	
2034893	c+	Lactose	3,0	AM	-	Cronobacter	P	AL	-	Cronobacter	P	AL	-	Cronobacter	PA	AL	-	Cronobacter	P	PA	
2034894	c+	Lecithin soy powder	3,0	AM	-	Cronobacter	P	BH	-	Cronobacter	P	BM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA	
2034895	c+	Powdered whole milk	<1	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA	
2066816	c+	Whey permeate	3,2	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA	
2066817	c+	Wheat flour	2,0	AM	-	Cronobacter	P	AM	-	Cronobacter	P	AM	-	Cronobacter	PA	AM	-	Cronobacter	P	PA	
2055441	c+	Rice flour batch 2	/	EM	/	/	A	BM	-	Cronobacter	P	BM	-	Cronobacter	PD	BM	-	Cronobacter	P	PD	

APPENDIX E

Relative level of detection raw results

Key:

- + : positive result / presence of typical colonies
- : negative result / absence of typical colonies
- / : test not realized
- st : absence of growth of any colony on the Petri dish
- (x) : number of typical colonies
- A : *Cronobacter* not detected
- P : *Cronobacter* detected

Bacterial load

L = low

M = medium

H = high

∅ = absence

Distribution of the microflora

A = pure culture of typical colonies

B = mix with a majority of typical colonies

C = mix with a minority of typical colonies

D = mix with rare typical colonies

E = absence of typical colonies

Infant formula 10 g

Infant formula with probiotics - Anaerobic lactic flora 5.5×10^5 CFU/g

Strain: *Cronobacter sakazakii* Ad 1418

Sample N°	Inoculation level / sample	ISO FDIS 22964				Cronobacter Precis				
		CCI	Confirmatory tests	Final result	Number positive samples / Total	CCI	Confirmation	Final result	CCI (streaking after subculture in CSB)	Number positive samples/ Total
3627	/	st	/	A	0/5	st	/	A	st	0/5
3628		st	/	A		st	/	A	st	
3629		st	/	A		st	/	A	st	
3630		st	/	A		st	/	A	st	
3631		st	/	A		st	/	A	st	
3632	4.1	st	/	A	12/20	st	/	A	st	12/20
3633		+p	+	P		+p	+	P	+p	
3634		st	/	A		st	/	A	st	
3635		+p	+	P		+p	+	P	+p	
3636		+p	+	P		+p	+	P	+p	
3637		+p	+	P		+p	+	P	+p	
3638		+p	+	P		+p	+	P	+p	
3639		+p	+	P		+p (2col)	+	P	+p	
3640		st	/	A		st	/	A	st	
3641		+p	+	P		+p	+	P	+p	
3642		+p	+	P		+p	+	P	+p	
3643		st	/	A		st	/	A	st	
3644		st	/	A		st	/	A	st	
3645		+p	+	P		+p	+	P	+p	
3646		st	/	A		st	/	A	st	
3647	+p	+	P	+p	+	P	+p			
3648	st	/	A	st	/	A	st			
3649	+p	+	P	+p	+	P	+p			
3650	st	/	A	st	/	A	st			
3651	+p	+	P	+p	+	P	+p			
3652	8.2	+p	+	P	5/5	+p	+	P	+p	5/5
3653		+p	+	P		+p	+	P	+p	
3654		+p	+	P		+p	+	P	+p	
3655		+p	+	P		+p	+	P	+p	
3656		+p	+	P		+p	+	P	+p	

Infant formula 10 g

Infant formula without probiotics - Aerobic mesophilic flora <10 CFU/g

Strain: *Cronobacter sakazakii* Ad 1418

Sample N°	Inoculation level / sample	ISO FDIS 22964				Cronobacter Precis				
		CCI	Confirmatory tests	Final result	Number positive samples / Total	CCI	Confirmation	Final result	CCI (streaking after subculture in CSB)	Number positive samples/ Total
4554	/	-	/	A	0/5	-	/	A	-	0/5
4555		-	/	A		-	/	A	-	
4556		-	/	A		-	/	A	-	
4557		-	/	A		-	/	A	-	
4558		-	/	A		-	/	A	-	
5050	3.5	+	+	P	15/20	+	+	P	+	15/20
5051		+	+	P		+	+	P	+	
5052		+	+	P		+	+	P	+	
5053		+	+	P		+	+	P	+	
5054		+	+	P		+	+	P	+	
5055		+	+	P		+	+	P	+	
5056		+	+	P		+	+	P	+	
5057		+	+	P		+	+	P	+	
5058		st	/	A		st	/	A	st	
5059		st	/	A		st	/	A	st	
5060		+	+	P		+	+	P	+	
5061		+	+	P		+	+	P	+	
5062		st	/	A		st	/	A	st	
5063		st	/	A		st	/	A	st	
5064		+	+	P		+	+	P	+	
5065		+	+	P		+	+	P	+	
5066		st	/	A		st	/	A	st	
5067		+	+	P		+	+	P	+	
5068		+	+	P		+	+	P	+	
5069		+	+	P		+	+	P	+	
4574	8.4	+	+	P	5/5	+	+	P	+	5/5
4575		+	+	P		+	+	P	+	
4576		+	+	P		+	+	P	+	
4577		+	+	P		+	+	P	+	
4578		+	+	P		+	+	P	+	

Environmental samples

Process water - Rinse water during milk powder production - Aerobic mesophilic flora: 6 CFU/ml

Strain: *Cronobacter turicensis* Ad 1445

Sample N°	Inoculation level / sample	ISO FDIS 22964				Cronobacter Precis				
		CCI	Confirmatory tests	Final result	Number positive samples / Total	CCI	Confirmation	Final result	CCI (streaking after subculture in CSB)	Number positive samples/ Total
4306	/	st	/	A	0/5	st	/	A	st	0/5
4307		st	/	A		st	/	A	st	
4308		st	/	A		st	/	A	st	
4309		st	/	A		st	/	A	st	
4310		st	/	A		st	/	A	st	
4311	0.7	+	+	P	9/20	+		P	+	9/20
4312		st	/	A		st	/	A	st	
4313		st	/	A		st	/	A	st	
4314		st	/	A		st	/	A	st	
4315		st	/	A		st	/	A	st	
4316		+	+	P		+		P	+	
4317		+	+	P		+		P	+	
4318		+	+	P		st	/	A	st	
4319		+	+	P		+		P	+	
4320		+	+	P		st	/	A	st	
4321		st	/	A		st	/	A	st	
4322		st	/	A		+		P	+	
4323		st	/	A		+		P	+	
4324		st	/	A		st	/	A	st	
4325		+	+	P		+		P	+	
4326	st	/	A	st	/	A	st			
4327	+	+	P	+		P	+			
4328	st	/	A	+		P	+			
4329	+	+	P	st	/	A	st			
4330	st	/	A	st	/	A	st			
4331	1.9	+	+	P	4/5	+		P	+	3/5
4332		st	/	A		+		P	+	
4333		+	+	P		st	/	A	st	
4334		+	+	P		st	/	A	st	
4335		+	+	P		+		P	+	

Infant formula 375 g

Infant cereals with probiotics - Anaerobic lactic flora 5.4 x 10⁶ CFU/g

Strain: *Cronobacter dublinensis* GVV828

Sample N°	Inoculation level / sample	EN ISO 22964 (Ø)				Cronobacter Precis				
		CCI	Confirmatory tests	Final result	Number positive samples / Total	CCI	Confirmation	Final result	CCI (streaking after subculture in CSB)	Number positive samples / Total
2045429	/	Ø	/	A	0/5	EL	/	A	Ø	0/5
2045430		Ø	/	A		Ø	/	A	Ø	
2045431		Ø	/	A		EL	/	A	Ø	
2045432		Ø	/	A		Ø	/	A	Ø	
2045433		Ø	/	A		Ø	/	A	Ø	
2045409	2,0	AM	+	P	13/20	AM	+	P	AL	12/20
2045410		Ø	/	A		Ø	/	A	Ø	
2045411		AM	+	P		AM	+	P	AL	
2045412		AM	+	P		AH	+	P	AL	
2045413		AM	+	P		AM	+	P	AL	
2045414		AM	+	P		Ø	/	A	Ø	
2045415		AM	+	P		AM	+	P	AM	
2045416		Ø	/	A		AM	+	P	AM	
2045417		AM	+	P		Ø	/	A	Ø	
2045418		AM	+	P		AM	+	P	AM	
2045419		Ø	/	A		AM	+	P	AM	
2045420		Ø	/	A		Ø	/	A	Ø	
2045421		AM	+	P		AM	+	P	AM	
2045422		AM	+	P		Ø	/	A	Ø	
2045423		Ø	/	A		AM	+	P	AM	
2045424		AM	+	P		AM	+	P	AM	
2045425		Ø	/	A		AM	+	P	AM	
2045426		AM	+	P		Ø	/	A	Ø	
2045427		AM	+	P		Ø	/	A	Ø	
2045428		Ø	/	A		Ø	/	A	Ø	
2045434	6,0	AM	+	P	5/5	AM	+	P	AM	5/5
2045435		AM	+	P		AM	+	P	AM	
2045436		AM	+	P		AM	+	P	AM	
2045437		AM	+	P		AM	+	P	AM	
2045438		AM	+	P		AM	+	P	AM	

APPENDIX F - Exclusivity raw results

#	Strain	Code	Origin	Concentration SM (CFU/ml)	TSA	CCI	Typical colony (Yes/No)	Identification
1	<i>Citrobacter youngae</i>	YCF284	Ground beef	4,7 10 ⁵	+	cream colonies with black center	No	/
2	<i>Citrobacter brakii</i>	ARP296	Marinated pork	9,6 10 ⁵	+	cream colonies	No	/
3	<i>Citrobacter freundii</i>	YBR641	Pond water	1,1 10 ⁶	+	cream colonies with black center	No	/
4	<i>Enterobacter asburiae</i>	AAV895	Infant formula	9,9 10 ⁵	+	cream colonies	No	/
5	<i>Enterobacter cloacae</i>	VBT249	Lactoserum	9,6 10 ⁵	+	cream colonies	No	/
6	<i>Enterobacter hormachei</i>	WDP406	Leaven	7,7 10 ⁶	+	cream colonies	No	/
7	<i>Escherichia coli</i>	FBV114	Wipe cheese dairy factory basins	5,8 10 ⁶	+	cream colonies with blue reflection	No	ox : - ID32E: <i>E.coli</i>
8	<i>Escherichia coli</i>	XFX554	Thermised milk pressed cooked cheese	3,2 10 ⁶	+	cream colonies with blue reflection	No	ox : - ID32E: <i>E.coli</i>
9	<i>Escherichia coli</i>	FQN709	Infant formula	3,4 10 ⁶	+	cream colonies with blue reflection	No	ox : - ID32E: <i>E.coli</i>
10	<i>Hafnia alvei</i>	BEY899	Milk	1,1 10 ⁷	+	cream colonies with blue reflection	No	ox : - ID32E: <i>Hafnia alvei</i>
11	<i>Klebsiella oxytoca</i>	CGR888	Tiramisu	6,4 10 ⁶	+	cream colonies	No	/
12	<i>Klebsiella pneumoniae</i>	CEC296	Milk	8,0 10 ⁶	+	cream colonies	No	/
13	<i>Leclercia adecarboxylata</i>	DNX652	Infant formula	2,9 10 ⁶	+	cream colonies	No	/
14	<i>Salmonella enterica Napoli</i>	LDV630	Ewe raw milk cheese	3,0 10 ⁶	+	cream colonies with black center	No	/
15	<i>Salmonella enterica Dublin</i>	ZDP683	Raw milk cheese	6,4 10 ⁶	+	cream colonies with black center	No	/
16	<i>Serratia liquefaciens</i>	JAR249	Vanilla ice-cream	1,1 10 ⁷	+	cream colonies with blue reflection	No	ox : - ID32E: <i>Serratia sp.</i>
17	<i>Serratia marcescens</i>	BJK3652	Food	3,2 10 ⁶	+	cream colonies with black center	No	/
18	<i>Serratia odorifera</i>	XAL298	Marinated salmon	9,6 10 ⁶	+	∅	/	/
19	<i>Serratia fonticola</i>	XUP895	River water	7,2 10 ⁶	+	cream colonies with blue center	No	ox : - ID32E: <i>Serratia sp.</i>
20	<i>Yersinia enterocolitica</i>	RKL458	Whipped cream pastry	4,6 10 ⁶	+	∅	/	/
21	<i>Pantoea agglomerans</i>	LAS822	Gentian root extract	4,8 10 ⁶	+	∅	/	/
22	<i>Providencia burhodogranariae</i>	VRY654	Collection	5,6 10 ⁶	+	∅	/	/
23	<i>Raoultella ornithinolytica</i>	STV984	Waste treatment plant water	4,8 10 ⁶	+	cream colonies with blue center	No	ox : - ID32E: <i>Raoultella sp</i>
24	<i>Shigella flexneri</i>	WMH220	Collection	6,4 10 ⁶	+	cream colonies with blue center	No	ox : - ID32E: <i>Shigella sp</i>
25	<i>Enterobacter ludwigii</i>	JGQ964	Environment	7,6 10 ⁶	+	cream colonies	No	/
26	<i>Enterobacter cancerogenus</i>	JHV807	Environment	1,4 10 ⁷	+	cream colonies	No	/
27	<i>Enterobacter mori</i>	JKJ301	Environment	2,2 10 ⁷	+	cream colonies	No	/
28	<i>Enterobacter pyrinus</i>	JLE589	Environment	1,3 10 ⁷	+	small cream colonies	No	/
29	<i>Pluribacter gergoviae</i>	JMU884	Collection	1,0 10 ⁷	+	small cream colonies	No	/
30	<i>Franconibacter helveticus</i>	JMV126	Fruit powder	5,2 10 ⁶	+	blue to blue green colonies	Yes	ox : - ID32E: ∅ identification MALDI TOF: <i>Franconibacter sp</i>

APPENDIX F

Inclusivity strains

n°	Genus	Species	N°	Origin	Inoculation level CFU/225ml	Brilliance CCI	Confirmation
1	<i>Cronobacter</i>	<i>dublinensis</i>	DSM18705	Dairy Product	34	+	+
2	<i>Cronobacter</i>	<i>malonaticus</i>	DSM18702	Dairy Product	11	+	+
3	<i>Cronobacter</i>	<i>malonaticus</i>	Ad1708	Dairy Product	53	+	+
4	<i>Cronobacter</i>	<i>muytjensii</i>	CIP103581	/	31	+	+
5	<i>Cronobacter</i>	<i>sakazakii</i>	Ad939	Infant formula	42	+	+
6	<i>Cronobacter</i>	<i>sakazakii</i>	Ad940	Infant formula	120	+	+
7	<i>Cronobacter</i>	<i>sakazakii</i>	Ad941	Infant formula	67	+	+
8	<i>Cronobacter</i>	<i>sakazakii</i>	Ad942	Infant formula	89	+	+
9	<i>Cronobacter</i>	<i>sakazakii</i>	Ad943	Infant formula	28	+	+
10	<i>Cronobacter</i>	<i>sakazakii</i>	Ad944	Infant formula	31	+	+
11	<i>Cronobacter</i>	<i>sakazakii</i>	Ad945	Infant formula	36	+	+
12	<i>Cronobacter</i>	<i>sakazakii</i>	Ad946	Infant formula	50	+	+
13	<i>Cronobacter</i>	<i>sakazakii</i>	Ad947	Infant formula	46	+	+
14	<i>Cronobacter</i>	<i>sakazakii</i>	Ad948	Infant formula	47	+	+
15	<i>Cronobacter</i>	<i>sakazakii</i>	Ad949	Infant formula	71	+	+
16	<i>Cronobacter</i>	<i>sakazakii</i>	Ad950	Infant formula	49	+	+
17	<i>Cronobacter</i>	<i>sakazakii</i>	Ad951	Infant formula	41	+	+
18	<i>Cronobacter</i>	<i>sakazakii</i>	Ad952	Infant formula	37	+	+
19	<i>Cronobacter</i>	<i>sakazakii</i>	Ad953	Infant formula	29	+	+
20	<i>Cronobacter</i>	<i>sakazakii</i>	Ad963	Infant formula	18	+	+
21	<i>Cronobacter</i>	<i>sakazakii</i>	Ad704	Infant formula	26	+	+
22	<i>Cronobacter</i>	<i>sakazakii</i>	Ad831	Infant formula	12	+	+
23	<i>Cronobacter</i>	<i>sakazakii</i>	Ad829	Infant formula	23	+	+
24	<i>Cronobacter</i>	<i>sakazakii</i>	Ad916	Infant formula	17	+	+
25	<i>Cronobacter</i>	<i>sakazakii</i>	Ad893	Infant formula	26	+	+
26	<i>Cronobacter</i>	<i>sakazakii</i>	Ad894	Infant formula	14	+	+
27	<i>Cronobacter</i>	<i>sakazakii</i>	Ad895	Infant formula	28	+ (blue green)	+
28	<i>Cronobacter</i>	<i>sakazakii</i>	Ad896	Infant formula	28	+	+
29	<i>Cronobacter</i>	<i>sakazakii</i>	Ad897	Infant formula	2	+	+
30	<i>Cronobacter</i>	<i>sakazakii</i>	Ad898	Infant formula	17	+	+
31	<i>Cronobacter</i>	<i>dublinensis lactaridi</i>	DSMZ18707 T	Dairy Product	20	+	+
32	<i>Cronobacter</i>	<i>dublinensis lausannensis</i>	DSMZ 18706 T	Dairy Product	16	+	+
33	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1418	Infant formula	18	+	+
34	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1419	Infant formula	21	+	+
35	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1420	Infant formula	27	+	+
36	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1421	Infant formula	10	+	+
37	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1424	Infant formula	12	+	+
38	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1425	Infant formula	13	+	+
39	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1426	Infant formula	28	+	+
40	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1427	Infant formula	32	+	+
41	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1428	Infant formula	24	+	+
42	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1429	Infant formula	27	+	+
43	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1430	Infant formula	24	+	+
44	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1431	Infant formula	31	+	+
45	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1432	Infant formula	24	+	+
46	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1433	Infant formula	31	+	+
47	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1434	Infant formula	30	+	+
48	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1435	Infant formula	30	+	+

Microsept

n°	Genus	Species	N°	Origin	Inoculation level CFU/225ml	Brilliance CCI	Confirmation
49	<i>Cronobacter</i>	<i>turicensis</i>	Ad 1445	Infant formula	17	+	+
50	<i>Cronobacter</i>	<i>turicensis</i>	DSMZ 18703	/	15	+	+
51	<i>Cronobacter</i>	<i>malonaticus</i>	E752	Baby food	17	+	+
52	<i>Cronobacter</i>	<i>turicensis</i>	E681	Food	15	+	+
53	<i>Cronobacter</i>	<i>muytjensii</i>	E769	Milk powder	9	+	+
54	<i>Cronobacter</i>	<i>dublinensis</i> subsp <i>dublinensis</i>	LMG 23823T	Environment	20	+	+
55	<i>Cronobacter</i>	<i>dublinensis</i> subsp <i>lausanensis</i>	E798	/	22	+	+
56	<i>Cronobacter</i>	<i>universalis</i>	NCTC 9529T	water	31	+	+
57	<i>Cronobacter</i>	<i>condimenti</i>	LMG 26250T	Spiced meat	15	+	+

Appendix G

Results obtained by the collaborative laboratories and by the Expert Laboratory

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 x10⁴/g

N° Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
A2	-	/	/	/	-	-	-	/	-	NA
A6	-	/	/	/	-	-	-	/	-	NA
A8	-	/	/	/	-	-	-	/	-	NA
A11	-	/	/	/	-	-	-	/	-	NA
A15	-	/	/	/	-	-	-	/	-	NA
A19	-	/	/	/	-	-	-	/	-	NA
A22	-	/	/	/	-	-	-	/	-	NA
A23	-	/	/	/	-	-	-	/	-	NA
A3	+	+	-	/	+	+	+	<i>Hafnia alvei</i>	+	PA
A7	+	+	-	/	+	+	+	<i>Serratia maltophilia</i>	+	PA
A9	+	+	-	/	+	+	+	<i>Hafnia alvei/Serratia maltophilia</i>	+	PA
A13	-	/	/	/	-	-	-	/	-	NA
A16	+	+	-	/	+	+	+	<i>Hafnia alvei/Serratia maltophilia</i>	+	PA
A18	+	+	-	/	+	+	+	<i>E.coli</i>	+	PA
A21	+	+	-	/	+	+	+	<i>Hafnia alvei</i>	+	PA
A24	+	+	-	/	+	+	+	<i>E.coli</i>	+	PA
A1	+	+	-	/	+	+	+	<i>Hafnia alvei</i>	+	PA
A4	+	+	-	/	+	+	+	<i>Hafnia alvei</i>	+	PA
A5	+	+	-	/	+	+	+	<i>Serratia maltophilia</i>	+	PA
A10	+	+	-	/	+	+	+	<i>Hafnia alvei/Serratia maltophilia</i>	+	PA
A12	+	+	-	/	+	+	+	<i>Hafnia alvei/Serratia maltophilia</i>	+	PA
A14	+	+	-	/	+	+	+	<i>Hafnia alvei</i>	+	PA
A17	+	+	-	/	+	+	+	<i>E.coli</i>	+	PA
A20	+	+	-	/	+	+	+	<i>Hafnia alvei</i>	+	PA

Laboratory

B

Lactic flora: 8.3 x10⁴/g

N° Sample	Reference method: ISO/TS 22964					Alternative method			Agreement	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
B2	-	/	/	/	-	-	-	/	-	NA
B6	-	/	/	/	-	-	-	/	-	NA
B8	-	/	/	/	-	-	-	/	-	NA
B11	-	/	/	/	-	-	-	/	-	NA
B15	-	/	/	/	-	-	-	/	-	NA
B19	-	/	/	/	-	-	-	/	-	NA
B22	-	/	/	/	-	-	-	/	-	NA
B23	-	/	/	/	-	-	-	/	-	NA
B3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B7	-	/	/	/	-	-	-	/	-	NA
B9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B13	-	/	/	/	-	-	-	/	-	NA
B16	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B21	-	/	/	/	-	-	-	/	-	NA
B24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
B20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

C

Lactic flora: 8.4 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/CCI Agar	Biochemical galleries		
C2	-	/	/	/	-	-	-	/	-	NA
C6	-	/	/	/	-	-	-	/	-	NA
C8	-	/	/	/	-	-	-	/	-	NA
C11	-	/	/	/	-	-	-	/	-	NA
C15	-	/	/	/	-	-	-	/	-	NA
C19	-	/	/	/	-	-	-	/	-	NA
C22	-	/	/	/	-	-	-	/	-	NA
C23	-	/	/	/	-	-	-	/	-	NA
C3	-	/	/	/	-	-	-	/	-	NA
C7	-	/	/	/	-	-	-	/	-	NA
C9	-	/	/	/	-	-	-	/	-	NA
C13	-	/	/	/	-	-	-	/	-	NA
C16	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C18	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C21	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C24	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C1	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C4	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C5	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C10	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C12	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C14	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C17	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
C20	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA

Laboratory

D

Lactic flora: 8.3 x10⁴ /g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
D2	-	/	/	/	-	-	-	/	-	NA
D6	-	/	/	/	-	-	-	/	-	NA
D8	-	/	/	/	-	-	-/-*	/	-	NA
D11	-	/	/	/	-	+	+/-*	<i>Cronobacter sakazakii</i>	-	NA
D15	-	/	/	/	-	+	+/-*	<i>Cronobacter sakazakii</i>	-	NA
D19	-	/	/	/	-	-	-	/	-	NA
D22	+	+	-	<i>Cronobacter sakazakii</i>	+	-	-/-*	/	-	ND
D23	+	+	-	<i>Cronobacter sakazakii</i>	+	-	-/-*	/	-	ND
D3	-	/	/	/	-	-	-	/	-	NA
D7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D9	+	+	-	<i>E.coli</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D13	+	+	-	<i>Klebsiella ozeanae</i>	+	+	+	<i>E.coli</i>	+	PA
D16	+	+	-	<i>Serratia liquefaciens</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D18	-	/	/	/	-	-	-	/	-	NA
D21	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D24	+	+	-	<i>Cronobacter sakazakii</i>	+	-	-/-*	/	-	ND
D1	+	+	-	<i>Enterobacter aerogenes</i>	+	+	+	<i>E.coli</i>	+	PA
D4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D5	+	+	-	<i>E.coli</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D10	+	+	-	<i>Serratia liquefaciens</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D12	+	+	-	<i>E.coli</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
D20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Klebsiella ozeanae</i>	+	PA

*: second streaking

Laboratory

E

Lactic flora: 5.3 x10⁴/g

N° Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
E2	-	/	/	/	-	-	-	/	-	NA
E6	-	/	/	/	-	-	-	/	-	NA
E8	-	/	/	/	-	-	-	/	-	NA
E11	-	/	/	/	-	-	-	/	-	NA
E15	-	/	/	/	-	-	-	/	-	NA
E19	-	/	/	/	-	-	-	/	-	NA
E22	-	/	/	/	-	-	-	/	-	NA
E23	-	/	/	/	-	-	-	/	-	NA
E3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E13	-	/	/	/	-	-	-	/	-	NA
E16	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E18	-	/	/	/	-	-	-	/	-	NA
E21	-	/	/	/	-	-	-	/	-	NA
E24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
E20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

F

Lactic flora flora: 9.2 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method			Agreement	
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation				Final result
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
F2	-	/	/	/	-	-	-	/	-	NA
F6	-	/	/	/	-	-	-	/	-	NA
F8	-	/	/	/	-	-	-	/	-	NA
F11	-	/	/	/	-	-	-	/	-	NA
F15	-	/	/	/	-	-	-	/	-	NA
F19	-	/	/	/	-	-	-	/	-	NA
F22	-	/	/	/	-	-	-	/	-	NA
F23	-	/	/	/	-	-	-	/	-	NA
F3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F7	-	/	/	/	-	-	-	/	-	NA
F9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F13	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F16	-	/	/	/	-	-	-	/	-	NA
F18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F21	-	/	/	/	-	-	-	/	-	NA
F24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
F20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

G

Lactic flora: 1.9 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
G2	-	/	/	/	-	-	-	/	-	NA
G6	-	/	/	/	-	-	-	/	-	NA
G8	-	/	/	/	-	-	-	/	-	NA
G11	-	/	/	/	-	-	-	/	-	NA
G15	+	+	-	<i>Cronobacter sakazakii</i>	+	-	-	/	-	ND
G19	-	/	/	/	-	-	-	/	-	NA
G22	-	/	/	/	-	-	-	/	-	NA
G23	-	/	/	/	-	-	-	/	-	NA
G3	-	/	/	/	-	-	-	/	-	NA
G7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	-	+	+	PA
G9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	-	+	+	PA
G13	-	/	/	/	-	-	-	/	-	NA
G16	-	/	/	/	-	+	+	<i>Cronobacter sakazakii</i>	+	PD
G18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G21	-	/	/	/	-	-	-	/	-	NA
G24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G1	+	+	-	+	+	+	-	+	+	PA
G4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	-	+	+	PA
G5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
G20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

H

Lactic flora: 9.5 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
H2	-	/	/	/	-	-	-	/	-	NA
H6	-	/	/	/	-	-	-	/	-	NA
H8	-	/	/	/	-	-	-	/	-	NA
H11	-	/	/	/	-	-	-	/	-	NA
H15	-	/	/	/	-	-	-	/	-	NA
H19	-	/	/	/	-	-	-	/	-	NA
H22	-	/	/	/	-	-	-	/	-	NA
H23	-	/	/	/	-	-	-	/	-	NA
H3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H13	-	/	/	/	-	-	-	/	-	NA
H16	-	/	/	/	-	-	-	/	-	NA
H18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H21	-	/	/	/	-	-	-	/	-	NA
H24	-	/	/	/	-	-	-	/	-	NA
H1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
H20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

I

Lactic flora: 9.8 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
I2	-	/	/	/	-	-	-	/	-	NA
I6	-	/	/	/	-	-	-	/	-	NA
I8	-	/	/	/	-	-	-	/	-	NA
I11	-	/	/	/	-	-	-	/	-	NA
I15	-	/	/	/	-	-	-	/	-	NA
I19	-	/	/	/	-	-	-	/	-	NA
I22	-	/	/	/	-	-	-	/	-	NA
I23	-	/	/	/	-	-	-	/	-	NA
I3	-	/	/	/	-	-	-	/	-	NA
I7	-	/	/	/	-	-	-	/	-	NA
I9	-	/	/	/	-	-	-	/	-	NA
I13	-	/	/	/	-	-	-	/	-	NA
I16	+	+	-	<i>Klebsiella ozeanae</i>	+	+	+	<i>Serratia marcescens</i>	+	PA
I18	-	/	/	/	-	-	-	/	-	NA
I21	+	+	-	<i>Klebsiella ozeanae</i>	+	+	+	<i>C.lapagei</i>	+	PA
I24	+	+	-	<i>Serratia maltophilia</i>	+	+	+	<i>Serratia marcescens</i>	+	PA
I1	+	+	-	<i>Serratia maltophilia</i>	+	+	+	<i>Serratia maltophilia</i>	+	PA
I4	+	+	-	<i>Serratia maltophilia</i>	+	+	+	<i>Serratia maltophilia</i>	+	PA
I5	+	+	-	<i>Serratia maltophilia</i>	+	+	+	<i>Serratia maltophilia</i>	+	PA
I10	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>C.lapagei</i>	+	PA
I12	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Hafnia alvei</i>	+	PA
I14	+	+	-	<i>C.lapagei</i>	+	+	+	<i>Serratia maltophilia</i>	+	PA
I17	+	+	-	<i>Serratia maltophilia</i>	+	+	+	<i>Serratia marcescens</i>	+	PA
I20	+	+	-	<i>Hafnia alvei</i>	+	+	+	<i>Cedecea spp</i>	+	PA

I13*: sample leaked

Laboratory

J

Lactic flora: 5.1 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
J2	-	/	/	/	-	-	-	/	-	NA
J6	-	/	/	/	-	-	-	/	-	NA
J8	-	/	/	/	-	-	-	/	-	NA
J11	-	/	/	/	-	-	-	/	-	NA
J15	-	/	/	/	-	-	-	/	-	NA
J19	-	/	/	/	-	-	-	/	-	NA
J22	-	/	/	/	-	-	-	/	-	NA
J23	-	/	/	/	-	-	-	/	-	NA
J3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J7	-	/	/	/	-	-	-	/	-	NA
J9	-	/	/	/	-	-	-	/	-	NA
J13	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J16	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J21	-	/	/	/	-	-	-	/	-	NA
J24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
J20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

K

Lactic flora: 9.4 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
K2	-	/	/	/	-	-	-	/	-	NA
K6	-	/	/	/	-	-	-	/	-	NA
K8	-	/	/	/	-	-	-	/	-	NA
K11	-	/	/	/	-	-	-	/	-	NA
K15	-	/	/	/	-	-	-	/	-	NA
K19	-	/	/	/	-	-	-	/	-	NA
K22	-	/	/	/	-	-	-	/	-	NA
K23	-	/	/	/	-	-	-	/	-	NA
K3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K9	-	/	/	/	-	-	-	/	-	NA
K13	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K16	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K21	-	/	/	/	-	-	-	/	-	NA
K24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
K20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

L

Lactic flora: 5.6 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
L2	-	/	/	/	-	-	-	/	-	NA
L6	-	/	/	/	-	-	-	/	-	NA
L8	-	/	/	/	-	-	-	/	-	NA
L11	-	/	/	/	-	-	-	/	-	NA
L15	-	/	/	/	-	-	-	/	-	NA
L19	-	/	/	/	-	-	-	/	-	NA
L22	-	/	/	/	-	-	-	/	-	NA
L23	-	/	/	/	-	-	-	/	-	NA
L3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L13	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L16	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L21	-	/	/	/	-	-	-	/	-	NA
L24	-	/	/	/	-	-	-	/	-	NA
L1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA
L20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	/	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

M

Lactic flora: 1.5 x10⁵/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/CCI Agar	Biochemical galleries		
M2	-	/	/	/	-	-	-	/	-	NA
M6	-	/	/	/	-	-	-	/	-	NA
M8	-	/	/	/	-	-	-	/	-	NA
M11	-	/	/	/	-	-	-	/	-	NA
M15	-	/	/	/	-	-	-	/	-	NA
M19	-	/	/	/	-	-	-	/	-	NA
M22	-	/	/	/	-	-	-	/	-	NA
M23	-	/	/	/	-	-	-	/	-	NA
M3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M7	-	/	/	/	-	-	-	/	-	NA
M9	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M13	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M16	-	/	/	/	-	-	-	/	-	NA
M18	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M21	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M24	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
M20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

N

Lactic flora: 5.2 x10⁴/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
						Confirmation			Final result	
	ESIA	TSA	oxidase	Biochemical galleries	Final result	CCI Agar	CSB/CCI Agar	Biochemical galleries		
N2	-	/	/	/	-	-	-	/	-	NA
N6	-	/	/	/	-	-	-	/	-	NA
N8	-	/	/	/	-	-	-	/	-	NA
N11	-	/	/	/	-	-	-	/	-	NA
N15	-	/	/	/	-	-	-	/	-	NA
N19	-	/	/	/	-	-	-	/	-	NA
N22	-	/	/	/	-	-	-	/	-	NA
N23	-	/	/	/	-	-	-	/	-	NA
N3	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N7	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N9	-	/	/	/	-	-	-	/	-	NA
N13	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N16	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N18	+	+	-	<i>Cronobacter sakazakii</i>	+	-	-	/	-	ND
N21	-	/	/	/	-	-	-	/	-	NA
N24	-	/	/	/	-	-	-	/	-	NA
N1	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N4	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N5	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N10	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N12	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N14	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N17	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
N20	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

0

Lactic flora: 1.2 x10⁵/g

N°Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/CCI Agar	Biochemical galleries		
02	-	/	/	/	-	-	-	/	-	NA
06	-	/	/	/	-	-	-	/	-	NA
08	-	/	/	/	-	-	-	/	-	NA
011	-	/	/	/	-	-	-	/	-	NA
015	-	/	/	/	-	-	-	/	-	NA
019	-	/	/	/	-	-	-	/	-	NA
022	-	/	/	/	-	-	-	/	-	NA
023	-	/	/	/	-	-	-	/	-	NA
03	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
07	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
09	-	/	/	/	-	-	-	/	-	NA
013	-	/	/	/	-	-	-	/	-	NA
016	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
018	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
021	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
024	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
01	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
04	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
05	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
010	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
012	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
014	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
017	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
020	+	+	-	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

Laboratory

P (ADRIA)

Lactic flora: 8.5 x10⁴/g

N° Sample	Reference method: ISO/TS 22964					Alternative method				Agreement
	ESIA	TSA	Oxidase	Biochemical galleries	Final result	Confirmation			Final result	
						CCI Agar	CSB/ CCI Agar	Biochemical galleries		
P2	-	/	/	/	-	-	-	/	-	NA
P6	-	/	/	/	-	-	-	/	-	NA
P8	-	/	/	/	-	-	-	/	-	NA
P11	-	/	/	/	-	-	-	/	-	NA
P15	-	/	/	/	-	-	-	/	-	NA
P19	-	/	/	/	-	-	-	/	-	NA
P22	-	/	/	/	-	-	-	/	-	NA
P23	-	/	/	/	-	-	-	/	-	NA
P3	-	/	/	/	-	-	-	/	-	NA
P7	-	/	/	/	-	-	-	/	-	NA
P9	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P13	-	/	/	/	-	-	-	/	-	NA
P16	-	/	/	/	-	-	-	/	-	NA
P18	-	/	/	/	-	-	-	/	-	NA
P21	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P24	-	/	/	/	-	-	-	/	-	NA
P1	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P4	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P5	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P10	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P12	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P14	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P17	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA
P20	+	+	+	<i>Cronobacter sakazakii</i>	+	+	+	<i>Cronobacter sakazakii</i>	+	PA

APPENDIX H

Protocol of the reference method:

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

Infant formula, infant cereals and ingredients:

10 g + 90 mL BPW (ac. ISO 6887 standards requirements)

Environmental samples¹:

1 swab + 10 mL BPW

or 1 sponge + 100 mL BPW or 10 g + 90 mL BPW

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, galleries)

APPENDIX I

Extension protocols of the alternative method

Cronobacter Precis™

Infant formula, infant cereals and related ingredients³

375 g + 1875 ml BPW + novobiocin (6 mg/L) for products containing probiotics

↓
18 - 24 h
at 34-38°C

Production environmental samples⁴

25 g + 225 ml BPW
Swab + 10 ml BPW
Sponge + 100 ml ml BPW

↓
20 - 26 h
at 34-38°C



Possibility to store for 72 h at 5°C ± 3°C except for infant formula with probiotics (10 g sample size)

↓
Streaking 10 µl onto Brilliance CCI Agar

↓
24 h ± 2 h at 37°C ± 1°C or 41.5C ± 1°C

↓
Typical colonies (blue green)

↓
Biochemical gallery (e.g. Microbact)

or

Tests of the ISO 22964 method

or

Appropriate ISO 16140-6:2019 validated confirmation method

³ ISO 6887 requirements

⁴ 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)

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	<i>Limosilactobacillus fermentum heredium</i> 1.1 10 ⁷ CFU/g	<i>C. turicensis</i>	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.6	-
a-	2428653	Cereals with cocoa	<i>B.lactis</i> 3,4 10 ⁵ CFU/g	<i>C. sakazakii</i>	XBG933	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.7	-
a-	2428654	Cereals with biscuit	<i>B.lactis</i> 3,6 10 ⁵ CFU/g	<i>C. sakazakii</i>	H2P119	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.9	-
a-	2428656	Cereals with rice	<i>B.lactis</i> 1 10 ⁷ CFU/g	<i>C. malonaticus</i>	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.6	-
a+	2483211	Infant formula 1st age	<i>Bifidobacterium lactis</i> 1.4 10 ⁷ CFU/g	<i>C. sakazakii</i>	JCT201	Milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2483212	Infant formula 1st age	<i>S.thermophilus</i> 7.7 10 ⁶ CFU/g	<i>C. turicensis</i>	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2483213	Infant formula 1st age	<i>Bifidobacterium infantis</i> 6.3 10 ⁵ CFU/g	<i>C. sakazakii</i>	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
a+	2483214	Infant formula 1st age	<i>Bifidobacterium lactis</i> 5.1 10 ⁷ CFU/g	<i>C. dublinensis</i>	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.6	+
a+	2483215	Infant formula 2nd age	<i>Lactobacillus reuteri</i> DSM17938 7.7 10 ⁶ CFU/g	<i>C. sakazakii</i>	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.8	+
a+	2483216	Infant formula 2nd age	<i>Bifidobacterium infantis</i> 5.8 10 ⁵ CFU/g	<i>C. sakazakii</i>	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2483218	Infant formula 2nd age	<i>Bifidobacterium lactis</i> 8.6 10 ⁵ CFU/g	<i>C. sakazakii</i>	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
a+	2483219	Infant formula 3rd age	<i>Lactobacillus reuteri</i> DSM17938 1.1 10 ⁷ CFU/g	<i>C. dublinensis</i>	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
a+	2483220	Infant formula 3rd age	<i>Lactobacillus fermentum heredium</i> CECT5716 1.10 ⁶	<i>C. sakazakii</i>	JCT201	Milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	+
a+	2428655	Cereals with quinoa, banana, prun	<i>B.lactis</i> 3,4 10 ⁵ CFU/g	<i>C. sakazakii</i>	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.1	+
a+	2498700	Cereals with quinoa, banana, prun	<i>B.lactis</i> 3,4 10 ⁵ CFU/g	<i>C. sakazakii</i>	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
b-	2428658	Organic infant formula 3rd age	/	<i>C. sakazakii</i>	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.8	-
b-	2428659	PIF 1st age batch 1	/	<i>C. turicensis</i>	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.2	-
b-	2428661	PIF baby croissance batch 1	/	<i>C. dublinensis</i>	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.3	-
b-	2428662	PIF baby croissance batch 2	/	<i>C. dublinensis</i>	GVV828	Collection strain	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.6	-
b-	2428664	Infant cereals biscuit	/	<i>C. sakazakii</i>	XBG933	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.7	-
b-	2428665	Infant cereals cocoa & wheat	/	<i>C. sakazakii</i>	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.1	-
b-	2428666	Infant cereals vanilla	/	<i>C. sakazakii</i>	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.1	-
b-	2428667	Infant cereals honey	/	<i>C. sakazakii</i>	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.7	-
b+	2428660	PIF 1st age batch 2	/	<i>C. sakazakii</i>	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.3	+
b+	2428663	Infant cereals brioche - cocoa	/	<i>C. mytjensi</i>	GVV884	Collection strain	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.5	+
b+	2428668	Infant cereals vanilla	/	<i>C. sakazakii</i>	HXA137	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
b+	2498707	Infant cereals cocoa & wheat	/	<i>C. sakazakii</i>	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
b+	2498708	Infant formula for 6 at 12 months old	/	<i>C. sakazakii</i>	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	/	<i>C. dublinensis</i>	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	/	<i>C. sakazakii</i>	HXN562	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	+
b+	2498732	Infant cereals biscuit	/	<i>C. sakazakii</i>	JCT201	Milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	3.0	+
b+	2498733	Infant formula for 6 at 12 months old	/	<i>C. sakazakii</i>	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.6	+
c-	2392612	Whole milk powder	/	<i>C. turicensis</i>	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	3.0	-
c-	2392617	Lactose powder	/	<i>C. sakazakii</i>	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.2	-
c-	2392618	Soy lecithin	/	<i>C. malonaticus</i>	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	-
c-	2483274	Powdered whole milk	/	<i>C. turicensis</i>	HZN090	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.4	-
c+	2392610	Rice flour	/	<i>C. dublinensis</i>	DSEL33	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
c+	2392613	Rye flour	/	<i>C. dublinensis</i>	DSEL33	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
c+	2392614	Wheat flour	/	<i>C. malonaticus</i>	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	+
c+	2392615	Maltodextrin	/	<i>C. sakazakii</i>	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.8	+
c+	2392616	Whey	/	<i>C. sakazakii</i>	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.8	+
c+	2483271	Whey	/	<i>C. sakazakii</i>	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
c+	2483272	Soy lecithin	/	<i>C. malonaticus</i>	HSE385	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	+
c+	2483275	Caseinate	/	<i>C. sakazakii</i>	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
c+	2483276	Skimmed milk powder	/	<i>C. sakazakii</i>	HYD216	Dairy environment	Seeding: Lyophilized strain, 2 weeks at 20°C	/	2.0	-
c+	2498709	Powdered whole milk	/	<i>C. sakazakii</i>	JGG241	Infant milk powder	Seeding: Lyophilized strain, 2 weeks at 20°C	/	1.3	+
a-	2483254	Residue maltodextrin	/	<i>C. sakazakii</i>	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	1.8	-
a-	2483255	Residue dextrose	/	<i>C. sakazakii</i>	HXX792	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	-
a+	2483251	Residue PIF w/o probiotics	/	<i>C. sakazakii</i>	HXX792	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.4	+
a+	2483252	Residues maltodextrin	/	<i>C. sakazakii</i>	H2P119	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.6	+
a+	2483253	Residues flour	/	<i>C. sakazakii</i>	JBU888	Dairy environment	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
a+	2483261	Dusts vacuum PIF w/o probiotic industry	/	<i>C. sakazakii</i>	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
a+	2483262	Dusts vacuum milk powder industry	/	<i>C. sakazakii</i>	HXA137	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+
a+	2483263	Dusts vacuum PIF industry	/	<i>C. dublinensis</i>	DSEL33	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
a+	2483264	Dust suction filter weighing powders	/	<i>C. sakazakii</i>	HXA137	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	2.2	+

Appendix J - Artificial contaminations

Type	#	Sample	Probiotics	Strain	Reference	Origin	Injury protocol	Injury measurement	Inoc. level CFU/sample	Result
a+	2483265	Vacuum cleaner dust spice industry	/	<i>C. sakazakii</i>	RLP329	Plant products	Seeding: Air-dried strain, 2 weeks at 20°C	/	3.0	+
b-	2483202	Rinsing water milk tank 1	/	<i>C. sakazakii</i>	HYD216	Dairy environment	Seeding 3 days at 3±2°C	/	2.0	-
b+	2483196	Process water Ligne 1 R1 KS1	/	<i>C. dublinensis</i>	DSEL33	Plant products	Seeding 3 days at 3±2°C	/	2.8	+
b+	2483197	Process water Ligne 1 R1 KS2	/	<i>C. sakazakii</i>	HZP119	Dairy environment	Seeding 3 days at 3±2°C	/	1.8	+
b+	2483198	Process water Ligne 2 R1	/	<i>C. turincensis</i>	HZN090	Dairy environment	Seeding 3 days at 3±2°C	/	2.0	+
b+	2483199	Dosing water ligne 2 R1	/	<i>C. sakazakii</i>	HXK792	Dairy environment	Seeding 3 days at 3±2°C	/	1.8	+
b+	2483200	Rinsing water ligne 1 barrel emptying	/	<i>C. sakazakii</i>	HZP119	Dairy environment	Seeding 3 days at 3±2°C	/	1.8	+
b+	2483201	Rinsing water ligne 1 potion	/	<i>C. malonaticus</i>	HSE385	Plant products	Seeding 3 days at 3±2°C	/	3.0	+
b+	2483203	Rinsing water milk tank 2	/	<i>C. sakazakii</i>	JFU886	Dairy environment	Seeding 3 days at 3±2°C	/	1.6	+
b+	2498734	Rinsing water TL+line TL-TR SAEQI	/	<i>C. sakazakii</i>	JFU886	Dairy environment	Seeding 3 days at 3±2°C	/	3.0	+
b+	2498735	Rinsing water TR-TR SEQI	/	<i>C. sakazakii</i>	JFP615	Dairy environment	Seeding 3 days at 3±2°C	/	2.8	+
b+	2498736	Rinsing water TR1 SAEQI	/	<i>C. sakazakii</i>	JAY280	Dairy environment	Seeding 3 days at 3±2°C	/	3.0	+
b+	2498737	Rinsing water TL+line TL-TR SAEQI	/	<i>C. sakazakii</i>	JFU886	Dairy environment	Seeding 3 days at 3±2°C	/	1.6	+
c-	2483188	Wipe robot carpet	/	<i>C. muytjensi</i>	GVW884	Collection strain	Spiking: 7 days pH = 4	0.5	2.2	-
c+	2483189	Wipe interior small tank before washing	/	<i>C. turincensis</i>	BTY537	Plant products	Spiking: 7 days pH = 4 then 8 minutes at 56°C	0.5	2.2	+
c+	2483190	Wipe dairy floor	/	<i>C. sakazakii</i>	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	/	<i>C. sakazakii</i>	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	/	<i>C. turincensis</i>	BTY537	Plant products	Spiking: 7 days pH = 4 then 8 minutes at 56°C	0.5	3.0	+
c+	2483193	Swab zone 3 robot sleeve	/	<i>C. malonaticus</i>	HSE385	Plant products	Spiking: 7 days pH = 4 then 8 minutes at 56°C	1.3	5.0	+
c+	2483194	Wipe floor milk powder	/	<i>C. muytjensi</i>	GVW884	Collection strain	Spiking: 7 days pH = 4	0.5	1.6	+
c+	2483195	Wipe milk powder bench	/	<i>C. sakazakii</i>	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	/	<i>C. sakazakii</i>	JAY280	Dairy environment	Spiking: 7 days pH = 4 then 4 minutes at 56°C	0.6	2.8	+
c+	2498726	Wipe cabinet AZOMP3	/	<i>C. sakazakii</i>	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	/	<i>C. sakazakii</i>	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	/	<i>C. sakazakii</i>	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	/	<i>C. sakazakii</i>	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

∅: 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 Cronobacter if $x \leq 5$

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	Probiotics	Contamination			Reference method ISO 22964 ^a														BPW 18h at 34-38°C										BPW 72h at 2-8°C													
				Strain	Conta.	Level CFU	CCI After CSB	Confirmatory tests		Final result	CCI 41,5°C	Confirmation			Final result	Agreement	CCI 37°C	Confirmation			Final result	Agreement	ISO conf.		CCI 41,5°C 72h - 2-8°C	CCI 37°C 72h - 2-8°C	CCI 41,5°C	Conf.	Final result	Agreement	CCI 37°C	Conf.	Final result	Agreement										
								Ox.	Galaxy			Ox.	Galaxy without purification	Galaxy with purification				Ox.	Galaxy without purification	Galaxy with purification			Ox.	Galaxy											CCI after CSB	Ox.	Galaxy							
a-	2483217	HIPP BIOLOGIQUE Combiotic 2	<i>Limosilactobacillus fermentum heredium</i> 1.1 10 ⁸ CFU/g	<i>C. turicensis</i>	HZN090	Lyophilisation	1.6	- (e)	/	/	/	A	- (e)	/	/	/	A (FN)	NA	+	(DM) 2 colonies	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(BM)	-	<i>Cronobacter sp</i>	- (EM)	+	(DM)	- (EM)	/	/	A	NA	- (EM)	/	/	A	NA		
a-	2428653	Cereals with cocoa	<i>B.lactis</i> 3,4 10 ⁷ CFU/g	<i>C. sakazakii</i>	XBG933	Air-dried	1.6	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	A	NA	- (e)	/	/	A	NA		
a-	2428654	Cereals with biscuit	<i>B.lactis</i> 3,6 10 ⁷ CFU/g	<i>C. sakazakii</i>	HZP119	Air-dried	2.0	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	A	NA	- (e)	/	/	A	NA		
a-	2428656	Cereals with rice	<i>B.lactis</i> 1 10 ⁷ CFU/g	<i>C. malonoticus</i>	HSE385	Air-dried	1.5	- (e)	/	/	/	A	- (EM)	/	/	/	A	NA	-	(EM)	/	/	/	A	NA	-	(EM)	/	/	/	- (EM)	- (EM)	- (EM)	/	/	A	NA	- (EM)	/	/	A	NA		
a-	2428599	Thickened formula infant milk for 6 months old	<i>Bifidobacterium lactis</i> 1.4 10 ⁷ CFU/g	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
a-	2428600	Infant milk as a substitute for breastfeeding for 6 at 12 months old	<i>Lactobacillus reuteri</i> DSM17938 7.7 10 ⁶ CFU/g	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
a-	2428601	Organic infant formula for 1 at 3 years old	<i>Bifidobacterium lactis</i> 5.1 10 ⁷ CFU/g	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
a-	2428602	Infant cereals with milk chocolate and biscuit flavor	<i>Bifidobacterium lactis</i> 3.4 10 ⁵ CFU/g	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
a-	2428603	Infant cereals with 5 cereals	<i>Bifidobacterium lactis</i> 3.5 10 ⁵ CFU/g	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
a-	2428605	Infant cereals with rice	<i>Bifidobacterium lactis</i> 1 10 ⁷ CFU/g	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
a+	2483211	NESTLE NIDAL Nidalgest 1	<i>Bifidobacterium lactis</i> 1.4 10 ⁷ CFU/g	<i>C. sakazakii</i>	JCT201	Lyophilisation	2.0	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PA	+	(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483212	GUIGOZ Pelargon 1	<i>S.thermophilus</i> 7.7 10 ⁶ CFU/g	<i>C. turicensis</i>	HZN090	Lyophilisation	2.0	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PA	+	(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483213	MODILAC Doucea 1	<i>Bifidobacterium infantis</i> 6.3 10 ⁶ CFU/g	<i>C. sakazakii</i>	JGG241	Lyophilisation	2.6	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PA	+	(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483214	BABYBIO Optima 1	<i>Bifidobacterium lactis</i> 5.1 10 ⁷ CFU/g	<i>C. dublinensis</i>	GVV828	Lyophilisation	1.6	- (e)	/	/	/	A	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PD	+	(AM)	<i>Cronobacter sp</i>	P	PD
a+	2483215	GUIGOZ Evolia 2	<i>Lactobacillus reuteri</i> DSM17938 7.7 10 ⁶ CFU/g	<i>C. sakazakii</i>	HXN562	Lyophilisation	1.8	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PA	+	(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483216	MODILAC Actigest 2	<i>Bifidobacterium infantis</i> 5.8 10 ⁶ CFU/g	<i>C. sakazakii</i>	HYD216	Lyophilisation	2.0	- (e)	/	/	/	A	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PD	+	(AM)	<i>Cronobacter sp</i>	P	PD
a+	2483218	BABYBIO Optima 2	<i>Bifidobacterium lactis</i> 8.6 10 ⁵ CFU/g	<i>C. sakazakii</i>	HXN562	Lyophilisation	2.4	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(CM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(BM)	-	<i>Cronobacter sp</i>	+	(BM)	+	(CM)	+	(BM)	<i>Cronobacter sp</i>	P	PA	+	(BM)	<i>Cronobacter sp</i>	P	PA
a+	2483219	GUIGOZ Optipro 3	<i>Lactobacillus reuteri</i> DSM17938 1.1 10 ⁷ CFU/g	<i>C. dublinensis</i>	GVV828	Lyophilisation	2.4	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(DM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(DM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(BM)	-	<i>Cronobacter sp</i>	+	(DM)	+	(DM)	+	(CM)	<i>Cronobacter sp</i>	P	PA	+	(BM)	<i>Cronobacter sp</i>	P	PA
a+	2483220	HIPP BIOLOGIQUE Combiotic 3	<i>Lactobacillus fermentum heredium</i> CECT5716 1.1 10 ⁶ CFU/g	<i>C. sakazakii</i>	JCT201	Lyophilisation	2.0	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(BM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(BM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(BM)	+	(BM)	<i>Cronobacter sp</i>	P	PA	+	(BM)	<i>Cronobacter sp</i>	P	PA
a+	2428655	Cereals with quinoa, banana, prun	<i>B.lactis</i> 3,4 10 ⁷ CFU/g	<i>C. sakazakii</i>	RLP329	Air-dried	2.0	- (e)	/	/	/	A	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PD	+	(AM)	<i>Cronobacter sp</i>	P	PD
a+	2498700	Cereals with quinoa, banana, prun	<i>B.lactis</i> 3,4 10 ⁷ CFU/g	<i>C. sakazakii</i>	JGG241	Lyophilisation	2.6	+	(AM)	-	<i>Cronobacter sp</i>	P	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+	(AM)	-	<i>Cronobacter sp</i>	+	(AM)	+	(AM)	+	(AM)	<i>Cronobacter sp</i>	P	PA	+	(AM)	<i>Cronobacter sp</i>	P	PA
b-	2428657	PIF Bledigest 2	/	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/	/	
b-	2428658	PIF Galia Bio croissance 3	/	<i>C. sakazakii</i>	HXN562	Lyophilisation	1.8	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/	/	
b-	2428659	PIF 1er âge batch 1	/	<i>C. turicensis</i>	HZN090	Lyophilisation	1.2	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/	/	
b-	2428661	PIF baby croissance batch 1	/	<i>C. dublinensis</i>	GVV828	Lyophilisation	1.4	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
b-	2428662	PIF baby croissance batch 2	/	<i>C. dublinensis</i>	GVV828	Lyophilisation	1.6	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
b-	2428664	Infant cereals biscuit	/	<i>C. sakazakii</i>	XBG933	Air-dried	1.8	- (e)	/	/	/	A	- (EM)	/	/	/	A	NA	-	(EM)	/	/	/	A	NA	-	(EM)	/	/	/	- (EM)	- (EM)	- (EM)	/	/	/	/	/	/	/	/	/		
b-	2428665	Infant cereals cocoa & wheat	/	<i>C. sakazakii</i>	RLP329	Air-dried	2.2	- (e)	/	/	/	A	- (EM)	/	/	/	A	NA	-	(EM)	/	/	/	A	NA	-	(EM)	/	/	/	- (EM)	- (EM)	- (EM)	/	/	/	/	/	/	/	/	/		
b-	2428666	Infant cereals vanilla	/	<i>C. sakazakii</i>	HYD216	Lyophilisation	1.2	- (EM)	/	/	/	A	- (EM)	/	/	/	A	NA	-	(EM)	/	/	/	A	NA	-	(EM)	/	/	/	- (EM)	- (EM)	- (EM)	/	/	/	/	/	/	/	/	/		
b-	2428667	Infant cereals honey	/	<i>C. sakazakii</i>	HYD216	Lyophilisation	1.8	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	- (e)	/	/	/	/	/	/	/	/	/		
b-	2428607	Infant formula for 0 at 6 months old batch 1	/	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	ø	/	/	A	NA	ø	/	/	A	NA		
b-	2428608	Infant formula for 6 at 12 months old	/	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	ø	/	/	A	NA	ø	/	/	A	NA		
b-	2428609	Infant formula for 0 at 6 months old batch 2	/	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/	/	A	NA	-	(e)	/	/	/	A	NA	-	(e)	/	/	/	- (e)	- (e)	ø	/	/	A	NA	ø	/	/	A	NA		
b-	2428610	Thickened formula infant milk for 6 at 12 months old	/	/	/	/	/	- (e)	/	/	/	A	- (e)	/	/																													

Type	#	Sample	Contamination				Reference method																	BPW															
							ISO 22964*				20h at 34-38°C													72h at 2-8°C															
			Strain	Conta.	Level CFU	CCI After CSB	Confirmatory tests			CCI 41.5°C	Confirmation			Final result	Agreement	CCI 37°C	Confirmation			ISO conf.			CCI 41.5°C 72h - 2-8°C	CCI 37°C 72h - 2-8°C	CCI 41.5°C	Conf.	Final result	Agreement	CCI 37°C	Conf.	Final result	Agreement							
							Ox.	Galery	Final result		Ox.	Galery without purification	Galery with purification				Ox.	Galery without purification	Galery with purification	Ox.	Galery	Galery											CCI after CSB	Ox.	Galery				
a-	2483254	Residues maltodextrin	<i>C. sakazakii</i>	JBU888	Air-dried	1.8	-(ø)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EL)	/	/	/	A	NA	-(ø)	/	/	/	-(EM)	-(EL)	-(EM)	/	A	NA	-(EM)	/	A	NA		
a-	2483255	Residue dextrose	<i>C. sakazakii</i>	HXX792	Air-dried	2.4	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(EL)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(EL)	-(ø)	/	A	NA	-(ø)	/	A	NA		
a-	2483256	Residue PIF w/o probiotics	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
a-	2483257	Residues maltodextrin	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
a-	2483258	Residues flour	/	/	/	/	-(EM)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	-(EM)	-(EM)	-(EM)	/	A	NA	-(EM)	/	A	NA		
a-	2483259	Residues maltodextrin	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
a-	2483260	Residue dextrose	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
a-	2483266	Dusts vacuum PIF w/o probiotic industry	/	/	/	/	-(EM)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	-(EM)	-(EM)	-(EM)	/	A	NA	-(EM)	/	A	NA		
a-	2483267	Dusts vacuum milk powder industry	/	/	/	/	-(ø)	/	/	/	A	-(EL)	/	/	/	A	NA	-(EL)	/	/	/	A	NA	-(ø)	/	/	/	-(EL)	-(EL)	-(EL)	/	A	NA	-(EL)	/	A	NA		
a-	2483268	Dusts vacuum PIF industry	/	/	/	/	-(EM)	/	/	/	A	-(EL)	/	/	/	A	NA	-(EL)	/	/	/	A	NA	-(ø)	/	/	/	-(EL)	-(EL)	-(EL)	/	A	NA	-(EL)	/	A	NA		
a-	2483269	Dust suction filter weighing powders	/	/	/	/	-(ø)	/	/	/	A	-(EL)	/	/	/	A	NA	-(EL)	/	/	/	A	NA	-(ø)	/	/	/	-(EL)	-(EL)	-(EL)	/	A	NA	-(EL)	/	A	NA		
a-	2483270	Vacuum cleaner dust spice industry	/	/	/	/	-(EM)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	-(EM)	-(EM)	-(EM)	/	A	NA	-(EM)	/	A	NA		
a+	2483251	Residue PIF w/o probiotics	<i>C. sakazakii</i>	HXX792	Air-dried	2.4	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483252	Residues maltodextrin	<i>C. sakazakii</i>	HZP119	Air-dried	2.6	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483253	Residues flour	<i>C. sakazakii</i>	JBU888	Air-dried	2.2	+(CM)	-	<i>Cronobacter sp</i>	P	+(CM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(CM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(CM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(CM)	+(CM)	+(CM)	+(CM)	<i>Cronobacter sp</i>	P	PA	+(CM)	<i>Cronobacter sp</i>	P	PA
a+	2483261	Dusts vacuum PIF w/o probiotic industry	<i>C. sakazakii</i>	RLP329	Air-dried	3.0	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483262	Dusts vacuum milk powder industry	<i>C. sakazakii</i>	HXA137	Air-dried	2.2	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(BL)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
a+	2483263	Dusts vacuum PIF industry	<i>C. dublinensis</i>	DSEL33	Air-dried	2.0	-(ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+(AM)	+(AM)	+(AM)	+(BL)	<i>Cronobacter sp</i>	P	PD	+(AM)	<i>Cronobacter sp</i>	P	PD
a+	2483264	Dust suction filter weighing powders	<i>C. sakazakii</i>	HXA137	Air-dried	3.0	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	/	/	A	ND	-(EL)	/	/	/	A	ND	-(ø)	/	/	/	-(ø)	-(EL)	-(EL)	/	A	ND	-(BL)	/	A	ND		
a+	2483265	Vacuum cleaner dust spice industry	<i>C. sakazakii</i>	RLP329	Air-dried	3.0	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
b-	2483202	Rinsing water milk tank 1	<i>C. sakazakii</i>	HYD216	Seeding	2.0	-(EM)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	A	NA	-(ø)	/	/	/	-(EM)	-(EM)	-(EM)	/	A	NA	-(EM)	/	A	NA		
b-	2498691	Process water Ligne 1 R1 KS1	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498692	Process water Ligne 1 R1 KS2	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498693	Process water Ligne 2 R1	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(EL)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(EL)	-(ø)	/	A	NA	-(EL)	/	A	NA		
b-	2498694	Dosing water ligne 2 R1	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498695	Rinsing water ligne 1 vide fût	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498696	Rinsing water ligne 1 potion	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498697	Rinsing water milk tank 1	/	/	/	/	-(ø)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	-(EM)	-(EM)	-(EM)	/	A	NA	-(EM)	/	A	NA		
b-	2498698	Rinsing water milk tank 2	/	/	/	/	-(ø)	/	/	/	A	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	A	NA	-(EM)	/	/	/	-(EM)	-(EM)	-(EM)	/	A	NA	-(EM)	/	A	NA		
b-	2498712	Rinsing water TL+line TL-TR SAEQI	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498713	Rinsing water TR-TR SEQI	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498714	Rinsing water TR1 SAEQI	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498715	Rinsing water TL+line TL-TR SAEQI	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498716	Rinsing water TR-TR SEQI	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b-	2498717	Rinsing water TR1 SAEQI	/	/	/	/	-(ø)	/	/	/	A	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	A	NA	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	NA	-(ø)	/	A	NA		
b+	2483196	Process water Ligne 1 R1 KS1	<i>C. dublinensis</i>	DSEL33	Seeding	2.8	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
b+	2483197	Process water Ligne 1 R1 KS2	<i>C. sakazakii</i>	HZP119	Seeding	1.8	+(AM)	-	<i>Cronobacter sp</i>	P	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PA	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PA	+(AM)	<i>Cronobacter sp</i>	P	PA
b+	2483198	Process water Ligne 2 R1	<i>C. turicensis</i>	HZN090	Seeding	2.0	-(ø)	/	/	A	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+(AM)	-	<i>Cronobacter sp</i>	<i>Cronobacter sp</i>	P	PD	+(AM)	+(AM)	+(AM)	+(AM)	<i>Cronobacter sp</i>	P	PD	+(AM)	<i>Cronobacter sp</i>	P	PD
b+	2483199	Dosing water ligne 2 R1	<i>C. sakazakii</i>	HXX792	Seeding	1.8	+(AM)	-	<i>Cronobacter sp</i>	P	-(ø)	/	/	/	A	ND	-(ø)	/	/	/	/	A	ND	-(ø)	/	/	/	-(ø)	-(ø)	-(ø)	/	A	ND	-(ø)	/	A	ND		

Appendix L
Relative Level of Detection - raw results

Total aerobic microflora :100 UFC/g

Sample	Strain	Product	Inoculation/level	Reference method				BPW					
				ISO 22964*				18h at 34-38°C					
				CCI After CSB	Confirmatory tests		Final result	Number positive samples/Total	CCI 37°C	Oxydase	Confirmation	Final result	Number positive samples/Total
	OX	Gallery											
2498654	Cronobacter sakazakii JBU888	Infant formula with probiotic <i>Lactobacillus reuteri</i> DSM 17938 - 1,1 10 ⁷ CFU/g	0	- (Ø)	/	/	A	0/5	- (Ø)	/	/	A	0/5
2498655				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498656				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498657				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498658				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498659			1.0	- (Ø)	/	/	A	6/20	- (Ø)	/	/	A	4/20
2498660				- (Ø)	/	/	A		+ (AL)	-	<i>Cronobacter sp.</i>	P	
2498661				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498662				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498663				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498664				+ (AM)	-	<i>Cronobacter sp.</i>	P		- (Ø)	/	/	A	
2498665				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498666				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498667				+ (AM)	-	<i>Cronobacter sp.</i>	P		- (Ø)	/	/	A	
2498668				+ (AM)	-	<i>Cronobacter sp.</i>	P		- (Ø)	/	/	A	
2498669				+ (AM)	-	<i>Cronobacter sp.</i>	P		- (Ø)	/	/	A	
2498670				- (Ø)	/	/	A		+ (AL) 8	-	<i>Cronobacter sp.</i>	P	
2498671				- (Ø)	/	/	A		+ (AM)	-	<i>Cronobacter sp.</i>	P	
2498672				- (Ø)	/	/	A		+ (AM)	-	<i>Cronobacter sp.</i>	P	
2498673			- (Ø)	/	/	A	- (Ø)	/	/	A			
2498674			+ (AM)	-	<i>Cronobacter sp.</i>	P	- (Ø)	/	/	A			
2498675			- (Ø)	/	/	A	- (Ø)	/	/	A			
2498676			+ (AM)	-	<i>Cronobacter sp.</i>	P	- (Ø)	/	/	A			
2498677			- (Ø)	/	/	A	- (Ø)	/	/	A			
2498678			- (Ø)	/	/	A	- (Ø)	/	/	A			
2498679			2.7	- (Ø)	/	/	A	1/5	- (Ø)	/	/	A	1/5
2498680				+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P	
2498681				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498682				- (Ø)	/	/	A		- (Ø)	/	/	A	
2498683	- (Ø)	/		/	A	- (Ø)	/		/	A			

Total aerobic microflora :200 UFC/surface

Strain	Product	Sample	Inoculation/ level	Reference method				BPW					
				CCI After CSB	Confirmatory tests		Final result	Number positive samples/Total	20h at 34-38°C			Final result	Number positive samples/Total
					OX	Gallery			CCI 37°C	Oxydase	Confirmation		
<i>Cronobacter sakazakii</i> HZN090	Stainless steel 1"x1"	2483221	0	- (Ø)	/	/	A	0/5	- (Ø)	/	/	A	0/5
		2483222		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483223		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483224		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483225		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483226	9100	+ (AM)	-	<i>Cronobacter sp.</i>	P	5/20	- (Ø)	/	/	A	5/20
		2483227		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483228		+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P	
		2483229		- (Ø)	/	/	A		+ (AM)	-	<i>Cronobacter sp.</i>	P	
		2483230		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483231		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483232		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483233		- (Ø)	/	/	A		+ (AM)	-	<i>Cronobacter sp.</i>	P	
		2483234		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483235		- (Ø)	/	/	A		+ (AM)	-	<i>Cronobacter sp.</i>	P	
		2483236		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483237		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483238		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483239		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483240		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483241		+ (AM)	-	<i>Cronobacter sp.</i>	P		- (Ø)	/	/	A	
		2483242		+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P	
		2483243		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483244		- (Ø)	/	/	A		- (Ø)	/	/	A	
		2483245		+ (AM)	-	<i>Cronobacter sp.</i>	P		- (Ø)	/	/	A	
2483246	1 000 000	+ (AM)	-	<i>Cronobacter sp.</i>	P	5/5	+ (AM)	-	<i>Cronobacter sp.</i>	P	5/5		
2483247		+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P			
2483248		+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P			
2483249		+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P			
2483250		+ (AM)	-	<i>Cronobacter sp.</i>	P		+ (AM)	-	<i>Cronobacter sp.</i>	P			