

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

Validation study according to the ISO 16140-2

iQ-Check *Cronobacter* spp. method

(Certificate number: BRD 07/23-01/13)

for the detection of *Cronobacter* spp. in infant formula
and infant cereals with or without probiotics including ingredients
(30 g and 375 g) and production environmental samples

Qualitative method

> Expert Laboratory:	ADRIA ZA Creac'h Gwen 29196 Quimper Cedex (France)
> For:	BIO-RAD 3 boulevard Raymond Poincaré 92430 Marnes la Coquette (France)

This report consists of 87 pages, including 7 appendices.

Only copies including the totality of this report are authorised.

Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol♦.

Version 0
04 September 2025



List of abbreviations	3
1 INTRODUCTION	5
2 METHOD PROTOCOLS	6
2.1 Alternative method	6
2.1.1 <i>Principle</i>	6
2.1.2 <i>Protocol</i>	7
2.1.3 <i>Restriction</i>	8
2.2 Reference method	8
2.3 Study design	8
3 INITIAL VALIDATION, EXTENSION/RENEWAL STUDIES: RESULTS	9
3.1 Method Comparison Study	9
3.1.1 <i>Sensitivity study</i>	9
3.1.2 <i>Relative level of detection</i>	21
3.1.3 <i>Inclusivity / exclusivity</i>	23
3.1.4 <i>Practicability</i>	25
3.2 Inter-laboratory Study	26
3.2.1 <i>Study organisation</i>	26
3.2.2 <i>Experimental parameters controls</i>	27
3.2.3 <i>Results analysis</i>	28
3.2.4 <i>Calculation and interpretation</i>	32
4 GENERAL CONCLUSION	36
> <i>Appendix 1 – Flow diagram of the alternative method: iQ-Check Cronobacter spp.</i>	37
> <i>Appendix 2 - Flow diagram of the reference method:</i>	39
> <i>Appendix 3 – Artificial contamination of samples</i>	40
> <i>Appendix 4 – Sensitivity study: raw data</i>	45
> <i>Appendix 5 – Relative level of detection study: raw data</i>	66
> <i>Appendix 6 – Inclusivity and exclusivity study: raw data</i>	69
> <i>Appendix 7 – Inter-laboratory study: results obtained by the collaborative laboratories and the expert laboratory</i>	74

LIST OF ABBREVIATIONS

Method & protocol

CFU	Colony Forming Units
ILS	Interlaboratory Study
MCS	Method Comparison Study
RLOD	Relative Level of Detection
RTC	Ready to cook
RTE	Ready to eat
RTRH	Ready to reheat
SE	Relative Sensitivity
SP	Relative Specificity
IC	Internal Control

Interpretation

AL	Acceptability Limit
alt	Alternative method
\bar{D}	Average difference
FN	False Negative results
FNR	False Negative Ratio
FP	False Positive results
FPR	False Positive Ratio
LOD	Limit of Detection
NA	Negative agreement
NA _{FN (alt)}	Negative Agreement due to false negative alternative-method results
ND	Negative Deviation
ND _{FN (alt)}	Negative Deviation due to false negative alternative-method results
PA	Positive Agreement
PA _{FP (alt)}	Positive Agreement due to false positive alternative-method results
PD	Positive deviation
PD _{FP (alt)}	Positive Deviation due to false positive alternative-method results
ref	Reference method
TNA	Total Negative Agreement
TND	Total Negative Deviation

Raw data

-	No typical colonies but presence of background microflora
(x)	Number of colonies in the plate
*	Dilution of the extract in case of inhibition according to the alternative protocol (1/10)
1/2	50% level of target analyte
d	Doubtful result
FDRS	Free DNA Removal Solution
i	inhibition
I.C.	Internal control
m	Minority level of target analyte
M	Majority level of target analyte
NC	non-characteristic colony
ne	New DNA extraction
NI	no identification
ni	not isolated colony
No amp	Non characteristic PCR curve
p	Pure culture level of target analyte
st	Plate without any colony
Bold typing	artificially inoculated samples

Quality Assurance documents related to this study can be consulted upon request from **BIO-RAD**.

The technical protocol and the result interpretation were carried out according to the ISO 16140-2 (2016), ISO 16140-2/A1 (2024) and the AFNOR technical rules (Revision 12).

Validation protocols	<ul style="list-style-type: none"> ▪ ISO 16140-1 (2016): Microbiology of the food chain - Method validation — <i>Part 1: Vocabulary</i> ▪ ISO 16140-2 (2016) & ISO 16140-2/A1 (2024): Microbiology of the food chain - Method validation — <i>Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method</i> ▪ AFNOR technical rules (Revision 12)
Reference methods[♦]	ISO 22964 (2017) - Microbiology of the food chain - Horizontal method for the detection of <i>Cronobacter</i> spp.
Alternative method	iQ-Check <i>Cronobacter</i> spp.
Scope	<ul style="list-style-type: none"> > Infant formula and infant cereals with or without probiotics including ingredients (30 g and 375 g sample sizes) > Production environmental samples
Certification organism	AFNOR Certification (http://nf-validation.afnor.org/)

[♦] Analyses performed according to the COFRAC accreditation (Accreditation Testing n°1-0144, scope available on www.cofrac.fr)

1 INTRODUCTION

The iQ-Check *Cronobacter* spp. method was validated in January 2013 for infant formula cereals and production and production environmental samples (Certificate number BRD 07/23-01/13) according to the ISO 16140 (2003) standard. The study was performed by ADRIA. Since this date, the following renewals and extensions were performed:

Date	Description	Reference method	Validation standard	Conducted by
January 2013	Initial validation	ISO 22964 (2006)	ISO 16140 (2003)	ADRIA
March 2013	Extension to validate the CFX Manager™ Software IDE version 2.0 and the iQ-Check Prep	/	/	Bio-Rad internal data
October 2013	Extension to validate the CFX Manager™ Software IDE version 2.1 and the iQ-Check Prep	/	/	Bio-Rad internal data
November 2014	Extension to validate the CFX Manager™ Software IDE version 2.2 and the iQ-Check Prep v2	/	/	Bio-Rad internal data
January 2017	Renewal study	ISO 22964 (2006)	ISO 16140-2 (2016)	ADRIA
May 2018	Extension to validate the CFX Manager™ Software IDE version 3.0 and the iQ-Check Prep v3	/	/	Bio-Rad internal data
March 2020	Renewal for Infant formula and infant cereals with and without probiotics (30 g sample size) and environmental samples	ISO 22964 (2017)	ISO 16140-2 (2016)	QLab
	Extension study for the infant formula and infant cereals with and without probiotics including ingredients: <ul style="list-style-type: none"> ▪ For a new enrichment protocol for 375 g sample size associated with the Easy lysis protocol. ▪ For the use of the iQ-Check Free DNA Removal Solution (FDRS) protocol associated with the Easy lysis protocol. ▪ For the use of a new Application Protocol File (APF). 	ISO 22964 (2017)	ISO 16140-2 (2016)	ADRIA
May 2021	Extension for the use of the iQ-Check Prep v4	/	/	Bio-Rad internal data

Date	Description	Reference method	Validation standard	Conducted by
<i>December 2022</i>	Extension for the use of the CFX Opus Deep Well and CFX Manager Software IDE v3.1	/	/	Bio-Rad internal data
<i>December 2024</i>	Renewal study	ISO 22964 (2017)	ISO 16140-2 (2016) & ISO 16140-2/A1 (2024)	ADRIA
<i>April 2025</i>	Extension for the use of a new version of CFX Maestro IDE software v4.0 for PCR detection (The data related to this study are available upon request from AFNOR Certification).	/	/	ADRIA

As a new version of the ISO 22964 was published in 2017 including major modifications, the renewal study was performed using the ISO 22964 (2017) and all the analyses were performed again. These analyses were subcontracted to **Q-Laboratories Inc** (1930 Radcliff Drive, Cincinnati, OH 45204)

2 METHOD PROTOCOLS

2.1 Alternative method

The flow diagram of the alternative method is provided in **Appendix 1**.

2.1.1 Principle

The iQ-Check *Cronobacter* spp. kit is tests based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for *Cronobacter* spp., as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the MiniOpticon™ or the CFX96™ and CFX OPUS systems.

PCR is a powerful technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies are called amplicons.

This test allows the detection of *Cronobacter* spp. in food products and environmental samples previously enriched by culture in Buffered peptone water supplemented or not with selective supplements. It includes the following 4 main steps:

- Enrichment,
- DNA extraction,
- Real-time PCR,
- Data analysis & interpretation.

The FDRS protocol (Free DNA Removal Solution) can be applied to remove free DNA from food and environmental enriched samples prior to PCR analysis. It is performed by a selected enzyme and its specific buffer under optimized conditions. The iQ-Check lysis buffer associated with thermal lysis inactivates the enzyme, allowing extraction from intact and living cells.

Two application protocol files (APF) can be applied:

- The classical iQ-Check APF (Application Protocol File) corresponds to a 1h50 min PCR run,
- The APF Fast to reduce the PCR run time down to 1h10 min.

2.1.2 Protocol

The different steps are described in Table 1.

Table 1 - Protocols

Step	Category 1: Infant formula and infant cereals with and without probiotics including ingredients	Category 2: Infant formula and infant cereals with and without probiotics including ingredients	Category 3: Production environmental samples
Enrichment	30 g + 270 mL BPW + 10 µg /mL vancomycin 20h ± 2h at 37°C ± 1°C Subculture: 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C	375 g + 1125 mL Pre-warmed (37°C) BPW with PIF supplement* 18 h to 26 h at 37°C ± 1°C	30 g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) 18 h ± 2 h at 37°C
FDRS protocol**	Applied on 100 µl of decanted enriched broth.		
DNA extraction	Easy extraction protocol using Deepwell plates on 100µL enriched sample or 100µL FDRS treated enriched sample		
PCR	On 5 µL DNA extract using the CFX96 Real-Time Thermocycler with the CFX Manager software IDE version 3.0		
Confirmation of PCR tests	Streaking 10 µL of last enriched sample (BPW) onto RAPID'Sakazakii Incubation 24 h ± 2 h at 44°C ± 1°C	Streaking 10 µL onto RAPID'Sakazakii Incubation 24 h ± 2 h at 44°C ± 1°C	Subculture of BPW in mLST (0.1 mL + 10 mL) for 24 h ± 2 h at 44°C ± 1°C Streaking 10 µL onto RAPID'Sakazakii Incubation 24 h ± 2 h at 44°C ± 1°C
Enrichment broth storage	48 h at 5°C ± 3°C	72 h at 5°C ± 3°C	48 h at 5°C ± 3°C

* Addition of α-amylase for infant cereals (0.1 g/L)

** Optional

2.1.3 Restriction

There is no restriction for use.

2.2 Reference method♦

For the previous studies, the reference method was the ISO/TS 22964 (2006): horizontal method for the detection of *Enterobacter sakazakii*.

For the renewal and extension studies, the reference method was the ISO 22964 (April 2017) - Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

The flow diagram is given in **Appendix 2**.

2.3 Study design

The study is **an unpaired study design** as the reference and the alternative methods have different enrichment procedures for the extension study except for the surface samples tested in the environmental samples category.

The reference method used in this validation study (MCS part) was tested using a 10 g test portion and a 1:10 dilution ratio. The alternative method was validated using a 30 g test portion with a 1:10 dilution ratio for both the food and environmental categories. For the food category (infant formula and infant cereals with or without probiotics including ingredients), the method was also validated using a 375 g test portion with a 1:4 dilution ratio.

♦ Analyses performed according to the COFRAC accreditation (Accreditation Testing n°1-0144, scope available on www.cofrac.fr)

3 INITIAL VALIDATION, EXTENSION/RENEWAL STUDIES: RESULTS

3.1 Method Comparison Study

The method comparison study is a study performed by the expert laboratory to compare the alternative method with the reference method.

The study was carried out on a variety of samples and strains representative of the most frequently encountered products. This does not constitute an exhaustive list of the different matrices in the scope.

For any comment on the alternative method, please contact AFNOR Certification by logging on to the web page <http://nf-validation.afnor.org/contact-2/>.

3.1.1 Sensitivity study

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

3.1.1.1 Number and nature of samples

As previously described, it was not possible to keep the data from the initial validation study; all the data presented in the report are data obtained in 2019 and 2020; 127 samples were tested for the renewal study (categories 1 and 3) and 66 samples for the extension study (category 2).

Four protocols were tested during the sensitivity study:

- Lysis without FDRS + PCR APF Classic,
- Lysis without FDRS + PCR APF Fast,
- Lysis with FDRS + PCR APF Classic,
- Lysis with FDRS + PCR APF Fast.

As all these protocols gave the same results, only one interpretation was done all along the report.

Taking into account all the categories, 193 samples were tested providing 105 positive and 88 negative results. The distribution per tested category and type is given in Table 2.

Table 2 – Distribution per tested category and type

Expert lab	Category		Type	Positive samples	Negative samples	Total	
Q-Lab	1	Infant formula and infant cereals with and without probiotics including ingredients (30 g sample size)	a	Infant formula and infant cereals without probiotics	11	9	20
			b	Infant formula and infant cereals with probiotics	12	8	20
			c	Ingredients (Maltodextrin, NFDM*, whey...)	14	6	20
			Total		37	23	60
ADRIA	2	Infant formula and infant cereals with and without probiotics and ingredients (375 g sample size)	a	Infant formula and infant cereals without probiotics	13	9	22
			b	Infant formula and infant cereals with probiotics	11	11	22
			c	Ingredients (Maltodextrin, NFDM, whey...)	10	12	22
			Total		34	32	66
Q-Lab	3	Production environmental samples	a	Process water	8	14	22
			b	Surface sampling	11	9	20
			c	Dusts	15	10	25
			Total		34	33	67
Total				105	88	193	

* NFDM: non-fat dry milk

3.1.1.2 Artificial contamination of samples

Artificial contaminations were done by seeding and spiking protocols. The artificial contaminations are presented in **Appendix 3**.

75 samples were artificially contaminated, using 16 different strains. 72 gave a positive result.

The repartition of the positive samples per inoculation protocol and inoculation level is given in Table 3.

Table 3 - Repartition of the positive samples per inoculation protocol and inoculation level

Category	Naturally contaminated	Artificially contaminated							Total
		Cross contamination	Spiking protocol			Seeding protocol			
			≤ 5 CFU	5 < x ≤ 10 CFU	10 < x < 30 CFU	≤ 3 CFU	3 < x ≤ 10 CFU	10 < x < 30 CFU	
1	15	0	3	0	0	5	14	0	37
2	1	0	0	0	0	33	0	0	34
3	17	0	8	0	0	9	0	0	34
Total	33	0	11	0	0	47	14	0	105
%	31.4	0.0	10.5	0.0	0.0	44.8	13.3	0.0	100

The number of naturally contaminated samples tested for categories 1 and 3 is relatively high compared to the number of samples usually observed. For the environmental samples, they are coming 2 different milk companies with a total of 3 locations and 1 spice company with 2 different locations. Therefore, there were a total of 5 different locations.

13.3% of the samples were contaminated between 3 or 5 CFU and 10 CFU, this is in agreement with the AFNOR technical rules.

31.4 % of the samples were naturally contaminated.

3.1.1.3 Protocols applied during the validation study

> Incubation times

- Infant formula with and without probiotics (30 g): 18h at 37°C ± 1°C and 3 h at 37°C ± 1°C.
- Infant formula and infant cereals with and without probiotics and ingredients (375 g sample size): 18 h at 37°C ± 1°C.
- Production environmental samples: 16 h at 37°C ± 1°C.

> Extraction protocols

Both extraction protocols were tested for the initial validation study. For this renewal and extension studies, only the Easy protocol was tested. The samples were tested with and without the FDRS protocol.

For the FDRS protocol, an incubation time of 15 min in the thermoshaker was applied.

> PCR tests

Two Application Protocol Files are available; both were tested during the study: APF Classic and APF Fast.

The CFX 96 Deepwell was used with software IDE V3.0.

> Confirmation protocols

The positive PCR results were confirmed by streaking 10 µl of the enriched sample (BPW) onto RAPID[®] *Sakazakii* plates.

For the purpose of the validation only, the typical colonies were confirmed by the tests described in the ISO method:

- Subculture of 1 to 5 typical colonies on non-selective agar plate (TSA),
- Biochemical tests: oxidase test and biochemical gallery; API ID 32E was used during the validation study.

The negative samples were confirmed using the protocol of the reference method: subculture in CSB for 24 h ± 2 h at 41.5°C ± 1°C before streaking onto CCI Agar incubated for 24 h ± 2h at 41.5°C ± 1°C.

> **Enrichment broth storage**

The enrichments broths were stored for 48 h at 5°C ± 3°C and tested again (extraction, PCR and confirmatory tests) for category 1 (infant formula with and without probiotics, 30 g sample size) and category 3 (environmental samples) and for 72 h at 5°C ± 3°C for the category 2 (infant formula and infant cereals with and without probiotics including ingredients, 375 g sample size).

> **Lactic flora enumeration**

For products with probiotics, lactic bacteria enumeration was carried out using MRS pH 5.7 incubated in anaerobic conditions for 72 h at 30°C ± 1°C, according to ISO 15214.

3.1.1.4 Test results

Raw data per category are given in **Appendix 4**. The results are given in Table 4.

Table 4 – Summary of results obtained with the reference and alternative methods (after confirmation) of all samples for each category

Category		Type	Evaluation	PA	PD	TND	TNA	
Qlab	1	APF	a	Unpaired	11	0	0	9
			b		12	0	0	8
			c		12	0	2	6
			Total		35	0	2	23
		APF with FDRS	a		11	0	0	9
			b		12	0	0	8
			c		12	0	2	6
			Total		35	0	2	23
		APF Fast	a		11	0	0	9
			b		12	0	0	8
			c		12	0	2	6
			Total		35	0	2	23
	APF Fast with FDRS	a		11	0	0	9	
		b		12	0	0	8	
		c		12	0	2	6	
		Total		35	0	2	23	

Category		Type		Evaluation	PA	PD	TND	TNA	
ADRIA	2	APF	a	Infant formula and infant cereals without probiotics	Unpaired	11	2	0	9
			b	Infant formula and infant cereals with probiotics		9	2	0	11
			c	Ingredients (Maltodextrin, NFDM, whey...)		5	5	0	12
			Total			25	9	0	32
		APF with FDRS	a	Infant formula and infant cereals without probiotics	Unpaired	11	2	0	9
			b	Infant formula and infant cereals with probiotics		9	2	0	11
			c	Ingredients (Maltodextrin, NFDM, whey...)		5	5	0	12
			Total			25	9	0	32
		APF Fast	a	Infant formula and infant cereals without probiotics	Unpaired	11	2	0	9
			b	Infant formula and infant cereals with probiotics		9	2	0	11
			c	Ingredients (Maltodextrin, NFDM, whey...)		5	5	0	12
			Total			25	9	0	32
	APF Fast with FDRS	a	Infant formula and infant cereals without probiotics	Unpaired	11	2	0	9	
		b	Infant formula and infant cereals with probiotics		9	2	0	11	
		c	Ingredients (Maltodextrin, NFDM, whey...)		5	5	0	12	
		Total			25	9	0	32	
Qlab	3	APF	a	Process water	Unpaired	8	0	0	14
			b	Surface sampling	Paired	11	0	0	9
			c	Dusts	Unpaired	15	0	0	10
			Total		34	0	0	33	
		APF with FDRS	a	Process water	Unpaired	8	0	0	14
			b	Surface sampling	Paired	11	0	0	9
			c	Dusts	Unpaired	15	0	0	10
			Total		34	0	0	33	
		APF Fast	a	Process water	Unpaired	8	0	0	14
			b	Surface sampling	Paired	11	0	0	9
			c	Dusts	Unpaired	15	0	0	10
			Total		34	0	0	33	
	APF Fast with FDRS	a	Process water	Unpaired	8	0	0	14	
		b	Surface sampling	Paired	11	0	0	9	
		c	Dusts	Unpaired	15	0	0	10	
		Total		34	0	0	33		
Total APF					94	9	2	88	
Total APF with FDRS					94	9	2	88	
Total APF Fast					94	9	2	88	
Total APF Fast with FDRS					94	9	2	88	

Paired evaluation: $TND = ND_{FN(alt)}$ $TNA = NA + PD_{FP(alt)}$
 Unpaired evaluation: $TND = ND + ND_{FN(alt)} + PA_{FP(alt)}$ $TNA = NA + NA_{FN(alt)} + PD_{FP(alt)}$

3.1.1.5 Calculation of relative trueness (RT), sensitivity (SE), false positive ratio (FPR) and false negative ratio (FNR) for the alternative method

The calculations are presented in Table 5.

Table 5 – Calculation of the relative trueness (RT), the sensitivity (SE), the false positive ratio (FPR) and the false negative ratio (FNR) for the alternative method

Category		Type	Evaluation	PA	PA _{FP (alt)}	NA	NA _{FN (alt)}	ND	ND _{FN (alt)}	PD	PD _{FP (alt)}	TND	TNA	SE alt %	SE ref %	RT %	FPR %	FNR	
Qlab	1	Infant formula and infant cereals with and without probiotics including ingredients (30 g sample size)	APF	a	Infant formula and infant cereals without probiotics	Unpaired	11	0	9	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000	
				b	Infant formula and infant cereals with probiotics		12	0	8	0	0	0	8	100.0%	100.0%	100.0%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		12	2	6	0	0	2	6	85.7%	100.0%	90.0%	33.3%	0.000	
				Total	35		2	23	0	0	2	23	94.6%	100.0%	96.7%	8.7%	0.000		
			APF with FDRS	a	Infant formula and infant cereals without probiotics		11	0	9	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000
				b	Infant formula and infant cereals with probiotics		12	0	8	0	0	0	8	100.0%	100.0%	100.0%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		12	2	6	0	0	2	6	85.7%	100.0%	90.0%	33.3%	0.000	
				Total	35		2	23	0	0	2	23	94.6%	100.0%	96.7%	8.7%	0.000		
			APF Fast	a	Infant formula and infant cereals without probiotics		11	0	9	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000
				b	Infant formula and infant cereals with probiotics		12	0	8	0	0	0	8	100.0%	100.0%	100.0%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		12	2	6	0	0	2	6	85.7%	100.0%	90.0%	33.3%	0.000	
				Total	35		2	23	0	0	2	23	94.6%	100.0%	96.7%	8.7%	0.000		
			APF Fast with FDRS	a	Infant formula and infant cereals without probiotics		11	0	9	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000
				b	Infant formula and infant cereals with probiotics		12	0	8	0	0	0	8	100.0%	100.0%	100.0%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		12	2	6	0	0	2	6	85.7%	100.0%	90.0%	33.3%	0.000	
				Total	35		2	23	0	0	2	23	94.6%	100.0%	96.7%	8.7%	0.000		
ADRIA	2	Infant formula and infant cereals with and without probiotics and ingredients (up to 375 g sample size)	APF	a	Infant formula and infant cereals without probiotics	Unpaired	11	0	9	0	0	2	9	100.0%	84.6%	90.9%	0.0%	0.000	
				b	Infant formula and infant cereals with probiotics		9	0	11	0	0	2	11	100.0%	81.8%	90.9%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		5	0	12	0	0	5	12	100.0%	50.0%	77.3%	0.0%	0.000	
				Total	25		0	32	0	0	9	32	100.0%	73.5%	86.4%	0.0%	0.000		
			APF with FDRS	a	Infant formula and infant cereals without probiotics		11	0	9	0	0	2	9	100.0%	84.6%	90.9%	0.0%	0.000	
				b	Infant formula and infant cereals with probiotics		9	0	11	0	0	2	11	100.0%	81.8%	90.9%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		5	0	12	0	0	5	12	100.0%	50.0%	77.3%	0.0%	0.000	
				Total	25		0	32	0	0	9	32	100.0%	73.5%	86.4%	0.0%	0.000		
			APF Fast	a	Infant formula and infant cereals without probiotics		11	0	9	0	0	2	9	100.0%	84.6%	90.9%	0.0%	0.000	
				b	Infant formula and infant cereals with probiotics		9	0	11	0	0	2	11	100.0%	81.8%	90.9%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		5	0	12	0	0	5	12	100.0%	50.0%	77.3%	0.0%	0.000	
				Total	25		0	32	0	0	9	32	100.0%	73.5%	86.4%	0.0%	0.000		
			APF Fast with FDRS	a	Infant formula and infant cereals without probiotics		11	0	9	0	0	2	9	100.0%	84.6%	90.9%	0.0%	0.000	
				b	Infant formula and infant cereals with probiotics		9	0	11	0	0	2	11	100.0%	81.8%	90.9%	0.0%	0.000	
				c	Ingredients (Maltodextrin, NFDM, whey...)		5	0	12	0	0	5	12	100.0%	50.0%	77.3%	0.0%	0.000	
				Total	25		0	32	0	0	9	32	100.0%	73.5%	86.4%	0.0%	0.000		

Category		Type	Evaluation	PA	PA _{FP (alt)}	NA	NA _{FN (alt)}	ND	ND _{FN (alt)}	PD	PD _{FP (alt)}	TND	TNA	SE alt %	SE ref %	RT %	FPR %	FNR		
Qlab	3	Production environmental samples	APF	a	Process water	Unpaired	8	0	14	0	0	0	14	100.0%	100.0%	100.0%	0.0%	0.000		
				b	Surface sampling	Paired	11	0	9	0	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000
				c	Dusts	Unpaired	15	0	10	0	0	0	0	0	10	100.0%	100.0%	100.0%	0.0%	0.000
				Total			34	0	33	0	0	0	0	0	33	100.0%	100.0%	100.0%	0.0%	0.000
		APF with FDRS	a	Process water	Unpaired	8	0	14	0	0	0	0	0	14	100.0%	100.0%	100.0%	0.0%	0.000	
			b	Surface sampling	Paired	11	0	9	0	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000	
			c	Dusts	Unpaired	15	0	10	0	0	0	0	0	10	100.0%	100.0%	100.0%	0.0%	0.000	
			Total			34	0	33	0	0	0	0	0	33	100.0%	100.0%	100.0%	0.0%	0.000	
		APF Fast	a	Process water	Unpaired	8	0	14	0	0	0	0	0	14	100.0%	100.0%	100.0%	0.0%	0.000	
			b	Surface sampling	Paired	11	0	9	0	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000	
			c	Dusts	Unpaired	15	0	10	0	0	0	0	0	10	100.0%	100.0%	100.0%	0.0%	0.000	
			Total			34	0	33	0	0	0	0	0	33	100.0%	100.0%	100.0%	0.0%	0.000	
	APF Fast with FDRS	a	Process water	Unpaired	8	0	14	0	0	0	0	0	14	100.0%	100.0%	100.0%	0.0%	0.000		
		b	Surface sampling	Paired	11	0	9	0	0	0	0	0	9	100.0%	100.0%	100.0%	0.0%	0.000		
		c	Dusts	Unpaired	15	0	10	0	0	0	0	0	10	100.0%	100.0%	100.0%	0.0%	0.000		
		Total			34	0	33	0	0	0	0	0	33	100.0%	100.0%	100.0%	0.0%	0.000		
	Total APF				94	2	88	0	0	0	9	0	2	88	98.1%	91.4%	94.3%	2.3%	0.000	
	Total APF with FDRS				94	2	88	0	0	0	9	0	2	88	98.1%	91.4%	94.3%	2.3%	0.000	
	Total APF Fast				94	2	88	0	0	0	9	0	2	88	98.1%	91.4%	94.3%	2.3%	0.000	
	Total APF Fast with FDRS				94	2	88	0	0	0	9	0	2	88	98.1%	91.4%	94.3%	2.3%	0.000	

A summary of the results is given in Table 6.

**Table 6 - Summary of results for the four PCR protocols
(APF, APF with FDRS, APF Fast, APF Fast with FDRS)**

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \times 100 \%$	98.1 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \times 100 \%$	91.4 %
Relative trueness	$RT = \frac{(PA + TNA)}{N} \times 100 \%$	94.3 %
False positive ratio for the alternative method	$FPR = \frac{PA_{FP(alt)} + PD_{FP(alt)}}{TNA} \times 100 \%$	2.3 %
False negative ratio for the alternative method	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	0.000

3.1.1.6 Analysis of discordant results

The negative deviations are given in Table 7 and the positive deviations in Table 8.

Two negative deviations were observed, all with naturally contaminated samples. For these two samples ($PA_{FP(alt)}$), positive PCR results were observed twice but it was not possible to confirm the presence of *Cronobacter* spp. in the enrichment broth.

Nine positive deviations were observed, one for naturally contaminated sample and eight for artificially contaminated samples.

No false negative result was observed during the study (FNR = 0 %).

The analyses of discordant results according to the ISO 16140-2/A1 (2024) is the following (See Table 9).

Table 9 - Analyses of discordant results

Category	Type	N+	TND	PD	Paired study				Unpaired study		Mixed study		
					TND - PD	AL	TND + PD	AL	TND - PD	AL	TND - PD	AL	
1 Infant formula and infant cereals with and without probiotics including ingredients (30 g sample size)	APF	a	Infant formula and infant cereals without probiotics	11	0	0				0		0	
		b	Infant formula and infant cereals with probiotics	12	0	0				0		0	
		c	Ingredients (Maltodextrin, NFDM, whey...)	14	2	0				2		2	
		Total		37	2	0				2	3	2	3
	APF with FDRS	a	Infant formula and infant cereals without probiotics	11	0	0				0		0	
		b	Infant formula and infant cereals with probiotics	12	0	0				0		0	
		c	Ingredients (Maltodextrin, NFDM, whey...)	14	2	0				2		2	
		Total		37	2	0				2	3	2	3
	APF Fast	a	Infant formula and infant cereals without probiotics	11	0	0				0		0	
		b	Infant formula and infant cereals with probiotics	12	0	0				0		0	
		c	Ingredients (Maltodextrin, NFDM, whey...)	14	2	0				2		2	
		Total		37	2	0				2	3	2	3
	APF Fast with FDRS	a	Infant formula and infant cereals without probiotics	11	0	0				0		0	
		b	Infant formula and infant cereals with probiotics	12	0	0				0		0	
		c	Ingredients (Maltodextrin, NFDM, whey...)	14	2	0				2		2	
		Total		37	2	0				2	3	2	3
2 Infant formula and infant cereals with and without probiotics and ingredients (up to 375 g sample size)	APF	a	Infant formula and infant cereals without probiotics	13	0	2				-2		-2	
		b	Infant formula and infant cereals with probiotics	11	0	2				-2		-2	
		c	Ingredients (Maltodextrin, NFDM, whey...)	10	0	5				-5		-5	
		Total		34	0	9				-9	3	-9	3
	APF with FDRS	a	Infant formula and infant cereals without probiotics	13	0	2				-2		-2	
		b	Infant formula and infant cereals with probiotics	11	0	2				-2		-2	
		c	Ingredients (Maltodextrin, NFDM, whey...)	10	0	5				-5		-5	
		Total		34	0	9				-9	3	-9	3
	APF Fast	a	Infant formula and infant cereals without probiotics	13	0	2				-2		-2	
		b	Infant formula and infant cereals with probiotics	11	0	2				-2		-2	
		c	Ingredients (Maltodextrin, NFDM, whey...)	10	0	5				-5		-5	
		Total		34	0	9				-9	3	-9	3
	APF Fast with FDRS	a	Infant formula and infant cereals without probiotics	13	0	2				-2		-2	
		b	Infant formula and infant cereals with probiotics	11	0	2				-2		-2	
		c	Ingredients (Maltodextrin, NFDM, whey...)	10	0	5				-5		-5	
		Total		34	0	9				-9	3	-9	3

Category	Type	N+	TND	PD	Paired study				Unpaired study		Mixed study		
					TND - PD	AL	TND + PD	AL	TND - PD	AL	TND - PD	AL	
3 Production environmental samples	APF	a Process water	8	0	0					0		0	
		b Surface sampling	11	0	0	0		0				0	
		c Dusts	15	0	0					0		0	
		Total	34	0	0	0	3	0	6	0	3	0	3
	APF with FDRS	a Process water	8	0	0					0		0	
		b Surface sampling	11	0	0	0		0				0	
		c Dusts	15	0	0					0		0	
		Total	34	0	0	0	3	0	6	0	3	0	3
	APF Fast	a Process water	8	0	0					0		0	
		b Surface sampling	11	0	0	0		0				0	
		c Dusts	15	0	0					0		0	
		Total	34	0	0	0	3	0	6	0	3	0	3
	APF Fast with FDRS	a Process water	8	0	0					0		0	
		b Surface sampling	11	0	0	0		0				0	
		c Dusts	15	0	0					0		0	
		Total	34	0	0	0	3	0	6	0	3	0	3
Total APF		105	2	9	/	/	/	/	/	/	-7	3	
Total APF with FDRS		105	2	9	/	/	/	/	/	/	-7	3	
Total APF Fast		105	2	9	/	/	/	/	/	/	-7	3	
Total APF Fast with FDRS		105	2	9	/	/	/	/	/	/	-7	3	

The observed values for (TND - PD) and (TND + PD) meet the acceptability limit for each individual category and for the three combined categories (calculated values \leq AL).

3.1.1.7 *Enrichment broth storage at 5 ± 3 °C*

No change was observed after the storage at 5 ± 3 °C.

The analysis of the discordant results is identical to Table 9.

The observed values for (TND - PD) and (TND + PD) meet the acceptability limit for each individual category and for the three combined categories (calculated values \leq AL).

3.1.1.8 *Confirmation*

The positive PCR results were confirmed by streaking the enriched BPW onto RAPID' *Sakazakii* plates.

Typical colonies onto RAPID' *Sakazakii* were observed for all the positive PCR results, except for two samples (No 3c and 15c). For these samples, doubtful colonies were observed, they were identified as *Enterobacter cloacae*.

All the typical colonies were identified as *Cronobacter* spp with the biochemical galleries. The colonies were tested by the iQ-Check *Cronobacter* spp. kit and negative PCR results were observed.

3.1.1.9 *PCR inhibition*

On the 1208 PCR tests applied, only 8 PCR gave inhibited results. Non-inhibited results were obtained thanks to a 1:10 dilution of the DNA extract (see Tables 10 and 11).

Table 10 – Samples with PCR inhibition

Samples No	Product	Before or after storage at 5±3°C	Protocol	PCR results		
				Cq	I.C. Cq	Final result
1a	Organic baby cereal	After storage for 48h	APF Classic with FDRS	-/29.64*	-/32.81*	i/+*
19b	Fat malabsorption problems - infant formula with probiotics	After storage for 48h	APF Classic with FDRS	-/30.11*	-/32.62*	i/+*
7746	Non-fat dry milk powder	Before storage	APF Classic	-/26.74*	-/32.07*	i/+*
			APF Classic with FDRS	-/27.47*	-/31.83*	i/+*
7747	Non-fat dry milk powder	Before storage	APF Classic	-/26.47*	-/32.11*	i/+*
			APF Fast	-/27.10*	-/32.23*	i/+*
		After storage for 72h	APF Classic with FDRS	-/21.49*	-/34.14*	i/+*
7753	Non-fat dry milk powder	Before storage	APF Classic	-/24.59*	-/32.52*	i/+*

*: 1:10 dilution of the DNA extract

Table 11 – Percentage of PCR inhibitions per extraction protocol and APF file

	Without FDRS		With FDRS	
	APF Classic	APF Fast	APF Classic	APF Fast
Number of PCR tests	302	302	302	302
Number of PCR inhibitions	3	1	4	0
% inhibition	1.0	0.3	1.3	0.0

3.1.2 Relative level of detection

The relative level of detection is the level of detection at $P = 0.50$ (LOD_{50}) of the alternative (proprietary) method divided by the level of detection at $P = 0.50$ (LOD_{50}) of the reference method.

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

$$RLOD = \frac{LOD_{Alt.}}{LOD_{Ref.}}$$

The relative detection level is the smallest number of culturable micro-organisms that can be detected in the sample in 50% of occasions by the alternative and reference methods.

3.1.2.1 Experimental design

Three (matrix/strain) pairs were analyzed by the reference method and by the alternative method (See Table 12):

The following protocol was applied:

- A negative control: 5 samples,
- A low contamination level providing fractional recovery data, with 20 replicates,
- A high contamination level, with 5 replicates.

A total plate count determination on each matrix was performed to estimate the total microbial load on the day of analysis.

Table 12 - Defined (matrix/strain) pairs for the RLOD determination

	Category	Matrix	Strain	Origin	Storage condition after inoculation, before analysis	Protocol	Study design
Q-Lab	Infant formula and infant cereals with and without probiotics including ingredients (30 g)	Infant cereals with probiotics	<i>Cronobacter sakazakii</i> QL 11007.9	Rice Flour	Seeding protocol with lyophilized strain storage for 2 weeks at ambient temperature	30 g + 290 mL BPW + vancomycin mL 18 h 37°C	Unpaired
ADRIA	Infant formula and infant cereals with and without probiotics including ingredients (up to 375 g sample size)	Infant formula with probiotics	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding protocol with lyophilized strain storage for 2 weeks at ambient temperature	375 g + 1125 mL pre-warmed (37°C) BPW + PIF supplement 18 h 37°C	
Q-Lab	Environmental samples (30 g or 30 mL or sample device)	Process water	<i>Cronobacter sakazakii</i> CCUG 28868	Dish Brush	Seeding 48 h at 5 ± 3°C	30 mL + 290 mL BPW 18 h 37°C	

3.1.2.2 Calculation and interpretation of the RLOD

The raw data are given in **Appendix 5**.

The RLOD calculations were performed using the Excel spreadsheet available at <http://standards.iso.org/iso/16140> - RLOD version 4 (2024-01-10).

The LOD₅₀ calculations were done using the Excel spreadsheet available at <http://standards.iso.org/iso/16140> POD-LOD calculation program - version 12, 2024-03-05. The RLOD and LOD₅₀ are given Table 13.

Table 13 – Presentation of RLOD before and after confirmation of the alternative method results and LOD₅₀

Category	(Matrix / strain) pair	RLOD		Level of detection at 50% (CFU / test portion)	
		Calculation results	AL	Reference method	Alternative method
1	Infant cereals with probiotics / <i>Cronobacter sakazakii</i> QL 11007.9	0.896 [0.403; 1.994]	2.5	1.5 [0.8; 2.9]	1.3 [0.7; 2.5]
2	Infant formula with probiotics / <i>Cronobacter sakazakii</i> Ad1418	0.904 [0.461; 1.770]		1.1 [0.6; 1.8]	0.9 [0.5; 1.6]
3	Process water / <i>Cronobacter sakazakii</i> CCUG 28868	1.108 [0.524; 2.344]		1.5 [0.8; 2.6]	1.7 [0.9; 3.0]
Combined results		0.967 [0.635; 1.473]		1.3 [0.9; 1.8]	1.2 [0.9;1.7]

Note : the LOD₅₀ have been recalculated according to the most recent spreadsheet available on the ISO website. No change was observed with the previous values.

3.1.2.3 Conclusion

The RLOD values (using the confirmed alternative method results) meet the acceptability limit of 2.5 for unpaired studies, for all matrix/strain pairs tested.

The LOD₅₀ varies from 1.1 to 1.5 CFU/test portion for the reference method and from 0.9 to 1.7 CFU/ test portion for the alternative method.

3.1.3 Inclusivity / exclusivity

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

3.1.3.1 Test protocols

> Inclusivity

For the initial validation study (2012), 52 target strains were tested. Cultures were performed in BHI medium, incubated at 37°C. Dilutions were done in order to inoculate between 10 to 100 cells/225 mL BPW. The broths were incubated for 16 h at 37°C. The alternative method protocol was then performed.

For the extension study, the inclusivity testing was performed again as the protocol dedicated to big samples sizes (category 2) is more selective than the protocol tested

for the initial validation study. Dilutions were done in order to inoculate between 10 to 100 cells/225 mL BPW + PIF supplement, the broths were incubated for 18 h at 37°C and the protocol of the alternative method was then applied.

The same strains were tested for both initial and extension studies

> **Exclusivity**

For the initial validation study, 31 non-target strains were tested. Cultures were performed in BHI medium, incubated at 37°C. Dilutions were done in order to inoculate 10⁵ cells/mL BPW. 20 h incubation time was used. The alternative method protocol was then performed.

3.1.3.2 *Results*

Raw data are given in **Appendix 6**.

> **Inclusivity**

For both studies (initial and extension), the 52 target strains showed positive results.

> **Exclusivity**

The 31 non-target strains showed negative results.

3.1.4 Practicability

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

Storage conditions, shelf-life and modalities of utilisation after first use	The kit must be stored between +2°C and 8°C. Reagents stored between +2°C and +8°C can be used until the expiration date indicated on the reagent tube (See page 5)			
Time to results	Negative samples			
	Steps	Reference Method	Alternative method	
			Infant formula (+ vancomycin)	Environmental samples
	Sampling	Day 0	Day 0	Day 0
	Extraction	/	Day 1	Day 1
	PCR	/	Day 1	Day 1
	mLST subculture	Day 1	/	/
	Streaking onto selective agar	Day 2	/	/
	Final negative result	Day 3	Day 1	Day 1
	Positive presumptive sample or positive sample			
	Steps	Reference Method	Alternative method	
			Infant formula (+ vancomycin)	Environmental samples
	mLST subculture	/	/	Day 1
	Streaking onto RAPID' <i>Sakazakii</i>	/	Day 1	Day 2
Reading RAPID' <i>Sakazakii</i>	/	Day 2	Day 3	
Streaking onto nutritive agar	Day 3	/	/	
Oxidase test	Day 5	/	/	
API 20E test	Day 5	/	/	
Final positive result	Day 6	Day 2	Day 3	
Common step with the reference method	The enrichment step is common with the reference method only for the environmental samples			

The screening of the positive and negative samples is done within one day with the iQ-Check *Cronobacter spp.* detection kit, while three days are required with the ISO 22964 method.

3.2 Inter-laboratory Study

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

3.2.1 Study organisation

Probiotic infant formula containing *Lb. reuteri* was contaminated with the wild *Cronobacter sakazakii* Ad 940 strain species. In order to facilitate the study, the infant formula was first homogenized in sterile water. Samples were sent to 13 laboratories.

Samples were inoculated and sent on Monday 19 March 2012, as described below:

- 24 labelled samples (30 ml) for *Cronobacter spp.* detection by iQ-Check *Cronobacter spp.* method,
- 24 labelled samples (30 ml) for *Cronobacter spp.* detection by the reference method ISO/TS 22964 (2006),
- 1 unlabelled milk sample for aerobic mesophilic flora enumeration by ISO 4833 method,
- 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 analyses.

The analyses were started on Wednesday 21 March 2012.

The targeted inoculation levels were:

- 0 CFU/30 g,
- 1 – 10 CFU/30 g,
- 5 – 50 CFU/30 g.

At least, each laboratory received 24 samples of 30 g, i.e. 8 samples per inoculation level and method.

Blinded samples (code is only known by the expert laboratory) were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing temperature probe was added to the package in order to register the temperature profile during the transport, package delivery.

Samples were shipped in 24 h to 48 h to the different laboratories. Sample temperature had to stay lower or equal to 8,4°C during transport, and between 0°C – 8,5°C at arrival.

Collaborative study laboratories and the expert laboratory carried out the analyses with the alternative and reference methods at day 2, except Lab J at day 3.

3.2.2 Experimental parameters controls

3.2.2.1 Strain stability and background microflora stability

Sample stability was performed by inoculating the matrix at 10 CFU/30 ml, 1 CFU/30 ml and 100 CFU/30 ml. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level. *Triplicata* were analysed, and the results are given in the Table 14.

Table 14 - Sample stability

	Detection /30 ml (ISO/TS 22964 reference method)						Enumeration (CFU/ml)		
	Low level inoculation			High level inoculation			Sample 1	Sample 2	Sample 3
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3			
Day 0	-	+	-	+	+	+	190	170	250
Day 1	+	+	-	+	+	+	66	91	74
Day 2	-	+	+	+	+	+	66	72	63

No evolution was observed during storage at 5°C ± 3°C.

3.2.2.2 Contamination levels

The contamination levels and the sample codification were the following (see Table 15).

Table 15 - Contamination levels

Level	Samples	Theoretical target level (b/30 ml)	True level (b/30 ml sample)	Low limit / 30 ml sample	High limit / 30 ml sample
Level 0	2 – 6 – 10 – 11 – 17 – 19 – 22 – 24	0	/	/	/
Low level	1 – 4 – 9 – 12 – 14 – 18 – 20 – 21	1	0.8	0.7	1.0
High level	3 – 5 – 7 – 8 – 13 – 15 – 16 – 23	20	20.6	17.9	23.7

3.2.2.3 Logistic conditions

Temperature conditions are given in Table 16.

Table 16 - Sample temperatures at receipt

Collaborators	Temperature measured at receipt by the sensor (°C)	Temperature measured at receipt (°C)	Day of receipt		Day of analysis
A	2.0	4.1	20/03/2012	09h30	21/03/2012
B	1.0	3.4	20/03/2012	14h00	21/03/2012
C	1.5	4.0	20/03/2012	15h30	21/03/2012
D	2.0	5.5	20/03/2012	15h50	21/03/2012
E	2.0	4.7	20/03/2012	10h00	21/03/2012
F	2.0	10.1 ¹	20/03/2012	14h00	21/03/2012
G	1.5	4.2	20/03/2012	11h40	21/03/2012
H	3.0	3.1	20/03/2012	11h15	21/03/2012
I	0.0	1.1	20/03/2012	10h30	21/03/2012
J	1.5	4.8	20/03/2012	12h00	22/03/2012
K	1.5	4.9	20/03/2012	13h00	21/03/2012
L	2.5	5.0	20/03/2012	11h10	21/03/2012
M	1.0	2.5	20/03/2012	13h30	21/03/2012

No problem was encountered during the transport or at receipt for the 13 collaborators. All the samples were delivered on time and in appropriate conditions. Temperatures during shipment and at receipt were all correct.

3.2.3 Results analysis

Raw data are provided in **Appendix 7**.

3.2.3.1 Expert laboratory results

The results obtained by the expert laboratory are given in Table 17.

¹ Temperature measured after the sample storage at 4°C

Table 17 – Results obtained by the expert Lab.

Level	Reference method	Alternative method	
		before confirmation	after confirmation
L0	0/8	0/8	0/8
L1	4/8	5/8	4/8
L2	8/8	8/8	8/8

All the results were in agreement between the reference and the alternative methods.

3.2.3.2 Results observed by the collaborative laboratories

> **Aerobic mesophilic flora enumeration**

Depending on the Lab results, the enumeration levels varied from 8 CFU/ml to 66 CFU/ml, except for Lab K which numerated 2.0×10^5 CFU/ml.

> **Cronobacter spp. detection**

13 collaborators participated to the study.

Fractional positive recovery results were observed at level L1, for all the labs. Many cross contaminations were observed:

The Lab J started the analyses at Day 3, at 7 h am, while all the others run the analyses in the afternoon of Day 2, as expected.

The reviewers of this study have been contacted. Taking into account that fractional recovery results are still observed at level L1, it was concluded to integrate the Lab J results in the study.

Cross contaminations were observed in the blank samples for many Labs, either with the reference and the alternative methods. They are mainly due to the fact that:

- the Labs used micropipettes to transfer the primary enrichment broth to the second step of the protocols,
- the Labs were not used to run PCR based assays.

The results obtained are provided in Table 18 (reference method) and Table 19 (alternative method).

Table 18 - Positive results by the reference method (ALL the collaborators)

Collaborators	Contamination level		
	L0	L1	L2
A	0	1	8
B	0	2	8
C	2	5	8
D	0	4	8
E	0	6	8
F	1	6	8
G	2	4	8
H	4	6	8
I	0	5	8
J	0	6	8
K	0	3	8
L	0	3	8
M	0	3	8
Total	P₀ = 9	P₁ = 54	P₂ = 104

Table 19 - Positive results (before and after confirmation) by the alternative method (ALL the collaborators)

Collaborators	Contamination level								
	L0			L1			L2		
	PCR result	Confirmation result	Final result	PCR result	Confirmation result	Final result	PCR result	Confirmation result	Final result
A	1	0	0	6	5	5	8	8	8
B	1	0	0	6	6	6	8	8	8
C	7	6	6	5	4	4	8	8	8
D	5	0	0	5	5	5	8	8	8
E	0	0	0	5	5	5	8	8	8
F	1	0	0	4	4	4	8	8	8
G	0	0	0	6	6	6	8	8	8
H	3	0	0	6	6	6	8	8	8
I	0	0	0	3	3	3	8	8	8
J	0	0	0	4	4	4	8	8	8
K	0	0	0	5	5	5	8	8	8
L	0	0	0	2	2	2	8	8	8
M	5	3	3	6	4	4	7	7	7
Total	P₀ = 23	C₀ = 9	CP₀ = 9	P₁ = 63	C₁ = 59	CP₁ = 59	P₂ = 103	C₂ = 103	CP₂ = 103

According to the AFNOR technical rules, it is possible to include the results from a collaborator with maximum one cross contamination (presumptive or confirmed result) at Level 0. For this study, 5 collaborators should be removed (C-D-G-H- M); in this case, only 8 data sets remain available. It was thus decided to keep among these 5 labs, the labs with the least contamination as it was done for the initial validation study:

- Lab D: 5 positive results not confirmed results with the alternative method,
- Lab G 2 positive results with the reference method.

Finally, the results from 10 Labs were used for interpretation: A, B, D, E, F, G, I, J, K and M.

3.2.3.3 Results of the collaborators retained for interpretation

The results obtained with the 10 labs kept for interpretation are presented in Table 20 (reference method) and Table 21 (alternative method).

**Table 20 - Positive results by the reference method
(Without Labs C, H and M)**

Collaborators	Contamination level		
	L0	L1	L2
A	0	1	8
B	0	2	8
D	0	4	8
E	0	6	8
F	1	6	8
G	2	4	8
I	0	5	8
J	0	6	8
K	0	3	8
L	0	3	8
Total	P₀ = 3	P₁ = 40	P₂ = 80

**Table 21 - Positive results (before and after confirmation)
by the alternative method (Without Labs C, H and M)**

Collaborators	Contamination level								
	L0			L1			L2		
	PCR result	Confirmation result	Final result	PCR result	Confirmation result	Final result	PCR result	Confirmation result	Final result
A	1	0	0	6	5	5	8	8	8
B	1	0	0	6	6	6	8	8	8
D	5	0	0	5	5	5	8	8	8
E	0	0	0	5	5	5	8	8	8
F	1	0	0	4	4	4	8	8	8
G	0	0	0	6	6	6	8	8	8
I	0	0	0	3	3	3	8	8	8
J	0	0	0	4	4	4	8	8	8
K	0	0	0	5	5	5	8	8	8
L	0	0	0	2	2	2	8	8	8
Total	P₀ = 8	C₀ = 0	CP₀ = 0	P₁ = 46	C₁ = 45	CP₁ = 45	P₂ = 80	C₂ = 80	CP₂ = 80

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 (See Table 22).

Table 22 - Percentage specificity

Specificity for the reference method	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) \times 100 \% =$	96.3 %
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) \times 100 \% =$	100.0 %

N: number of all L0 tests

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

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

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

Fractional positive results were obtained for the low levels (L1). This inoculation level was retained for calculation.

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 23.

Table 23 - Summary of results for all collaborators obtained with the reference alternative methods for Level 1

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	Alternative method positive (A+)	Positive Agreement (A+/R+) PA = 24	Positive Deviation (R-/A+) PD = 21
	Alternative method negative (A-)	Total Negative Deviation (A-/R+) TND = 16 (0 ND_{FN(alt)}; 0 PA_{FP(alt)})	Total Negative Agreement (A-/R-) TNA = 19 (0 NA_{FN(alt)}; 1 PD_{FP(alt)})

Based on the data summarized in Table 23, the values of sensitivity of the alternative and reference methods, as well as the relative trueness, false positive ratio and false negative ratio for the alternative method taking account the confirmations, are the following (See Table 24).

Table 24 - Sensitivity, relative trueness and false positive ratio percentages

		Level 1
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \times 100 \%$	73.8%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \times 100 \%$	65.6%
Relative trueness	$RT = \frac{(PA + TNA)}{N} \times 100 \%$	53.8%
False positive ratio for the alternative method	$FPR = \frac{PA_{FP(alt)} + PD_{FP(alt)}}{TNA} \times 100 \%$	5%
False negative ratio for the alternative method	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	0.000

3.2.4.3 Interpretation of trueness data

The negative deviations are listed in Table 25 for Level 1 and the positive deviations in Table 26 for Level 1.

Table 25 - Negative deviations for Level 1

Lab	Level	Sample n°	iQ Check <i>Cronobacter</i>	
			PCR result	Confirmation
A	1	A1	-	/
B	1	B20	-	/
D	1	D9	-	-
E	1	E18	-	/
	1	E20	-	/
F	1	F1	-	/
	1	F4	-	/
	1	F14	-	/
I	1	I1	-	/
	1	I9	-	/
	1	I21	-	/
J	1	J18	-	-
	1	J20	-	-
K	1	K18	-	-
L	1	L12	-	-
	1	L14	-	-

The presence of *Cronobacter* spp was not confirmed in the enrichment broths for the 16 samples in negative deviation.

Table 26 - Positive deviations for Level 1

Lab	Level	Sample n°	iQ Check <i>Cronobacter</i>	
			PCR result	Confirmation
A	1	A9	+	+
	1	A12	+	+
	1	A14	+	+
	1	A18	+	+
	1	A20	+	+
B	1	B1	+	+
	1	B4	+	+
	1	B9	+	+
	1	B12	+	+
	1	B14	+	+
D	1	D4	+	+
	1	D21	+	+
E	1	E4	+	+
F	1	F12	+	+
G	1	G1	+	+
	1	G9	+	+
I	1	I20	+	+
K	1	K1	+	+
	1	K14	+	+
	1	K20	+	+
L	1	L4	+	+

For an **unpaired study design**, the difference between (TND – PD) is calculated for the level(s) where fractional recovery is obtained (so L_1 and possibly L_2). The observed value found for (TND – PD) shall not be higher than the AL. The AL is defined as $[(TND - PD)_{max}]$ and calculated per level where fractional recovery is obtained as described below using the following three parameters:

$$(p+)_{ref} = \frac{P_{x(ref)}}{N_{x(ref)}}$$

where

$P_{x(ref)}$ = is number of samples with a positive result obtained with the reference method at level x (L_1 or L_2) for all laboratories

$N_{x(ref)}$ = is number of samples tested at level x (L_1 or L_2) with the reference method by all laboratories

$$(p+)_{alt} = \frac{CP_{x(alt)}}{N_{x(alt)}}$$

where

$CP_{x(alt)}$ = is number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 or L_2) for all laboratories

$N_{x(alt)}$ = is number of samples tested at level x (L_1 or L_2) with the alternative method by all laboratories

$$(TND - PD)_{max} = \sqrt{3N_{x(ref)} \times ((p+)_{ref} + (p+)_{alt} - 2((p+)_{ref} \times (p+)_{alt}))}$$

where

$N_{x(ref)}$ = is number of samples tested for level x (L_1 or L_2) with the reference method by all laboratories.

The AL is not met when the observed value is higher than the AL. When the AL is not met, investigations should be made (e.g. root cause analysis) in order to provide an explanation of the observed results. Based on the AL and the additional information, it is decided whether the alternative method is regarded as not fit for purpose. The reasons for acceptance of the alternative method when the AL is not met shall be stated in the study report.

In this study, fractional recovery was observed at Level 1. The calculations are the following, according to the ISO 16140-2:2016 and ISO 16140-2/A1:2024 (See Table 27).

Table 27 - Calculations

	Level 1
N_x	80
$(p+)_{ref}$	0.5
$(p+)_{alt}$	0.6
AL = (TND - PD) max	10.95
TND - PD	-5
Conclusion	TND-PD ≤ AL

The calculated values for TND-PD meet the acceptability limits defined in the ISO 16140-2:2016 and ISO 16140-2/A1:2024.

3.2.4.4 Evaluation of the LOD₅₀ and RLOD between laboratories

The LOD₅₀ was calculated using the Excel spreadsheet available at https://standards.iso.org/iso/16140/-2/ed-1/en/amd/1/PODL0D-interlab_ver2.xlsm.

The RLOD is the ratio of LODs of the alternative method and of the reference method calculated as follows: RLOD= LOD_{p;alt}/ LOD_{p; ref}.

The results are used only for information (see Table 28).

Table 28 - LOD₅₀ and RLOD

Method	LOD 50%	RLOD
Reference	0.877 [0.565;1.360]	0.765
Alternative	0.671 [0.474;0.949]	

4 GENERAL CONCLUSION

The **method comparison study conclusions** are:

- ☒ In the sensitivity study, 3 categories were tested: 2 food categories and the environmental samples. The protocol of the alternative method shows 9 positive deviations (PD) and 2 total negative deviations (TND) for the 3 categories tested. The TND - PD and TND + PD meet the acceptability limits (AL) whatever the categories, and as well for the 3 tested categories. No false negative result was observed during the study (FNR= 0%)
- ☒ The Relative Levels of Detection (RLOD) meet the AL fixed at 2.5 whatever the matrix/strain pairs tested.
- ☒ The inclusivity and exclusivity testing gave the expected results for the 52 target strains and the 31 non-target strains.
- ☒ The screening of the positive and negative samples is done within one day with the iQ-Check *Cronobacter* spp. detection kit, while three days are required with the ISO 22964 method.
- ☒ It is possible to store the primary enrichment broth for 48 h at $5 \pm 3^{\circ}\text{C}$ for category 1 (Infant formula and infant cereals with and without probiotics including ingredients - 30 g sample size) and category 3 (environmental samples), and for 72h at $5 \pm 3^{\circ}\text{C}$ for category 2 (infant formula and infant cereals with and without probiotics including ingredients - 375 g sample size).

The **inter-laboratory study conclusions** are:

- ☒ The data and interpretations comply with the ISO 16140-2:2016, ISO 16140-2/A1:2024 requirements and AFNOR technical rules (Revision 12). **The iQ-Check *Cronobacter* spp. method is considered equivalent to the ISO standard.**

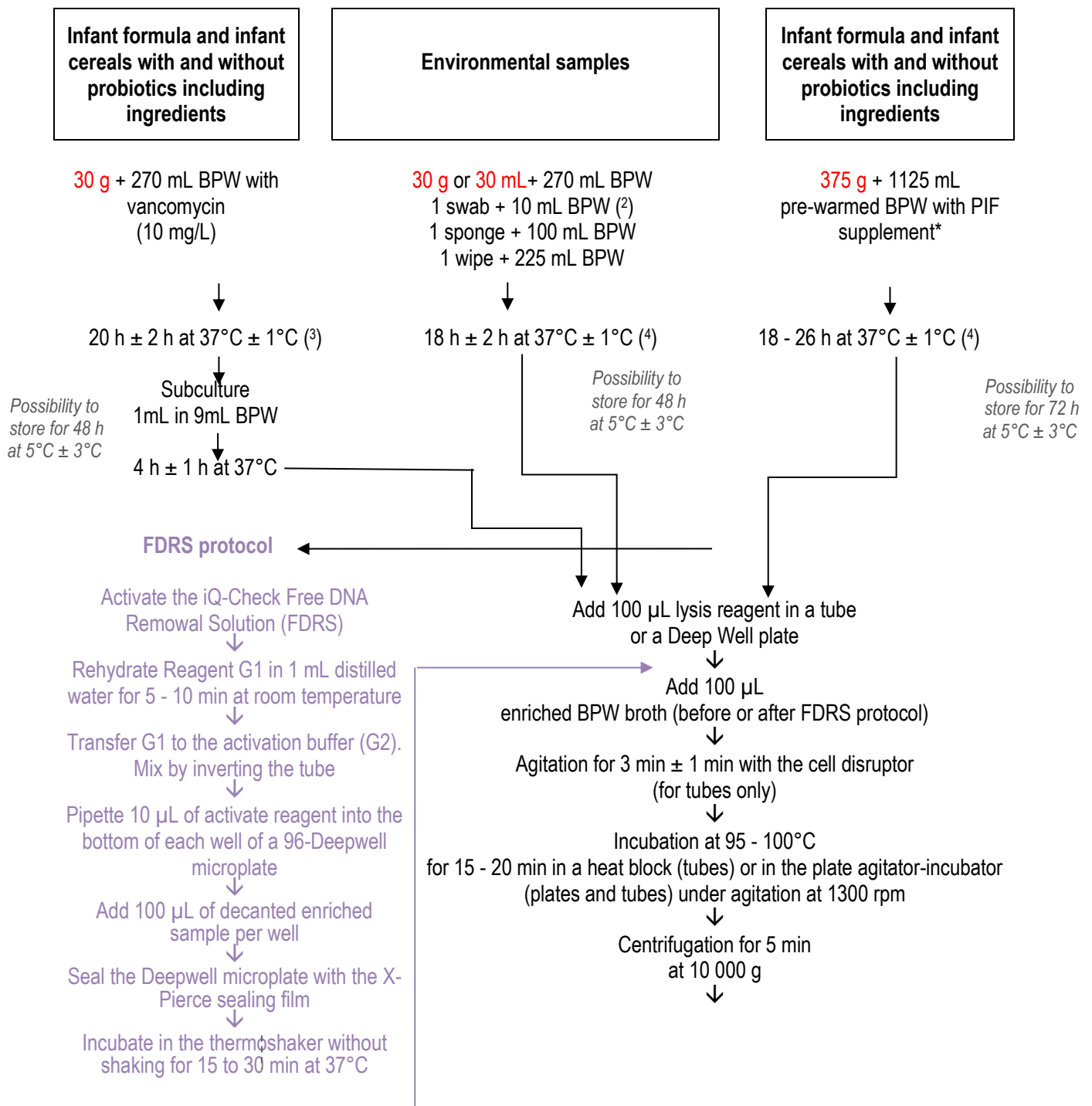
Quimper, 04 September 2025

Astrid CARIOU
Manager
Method performance in food microbiology



I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).

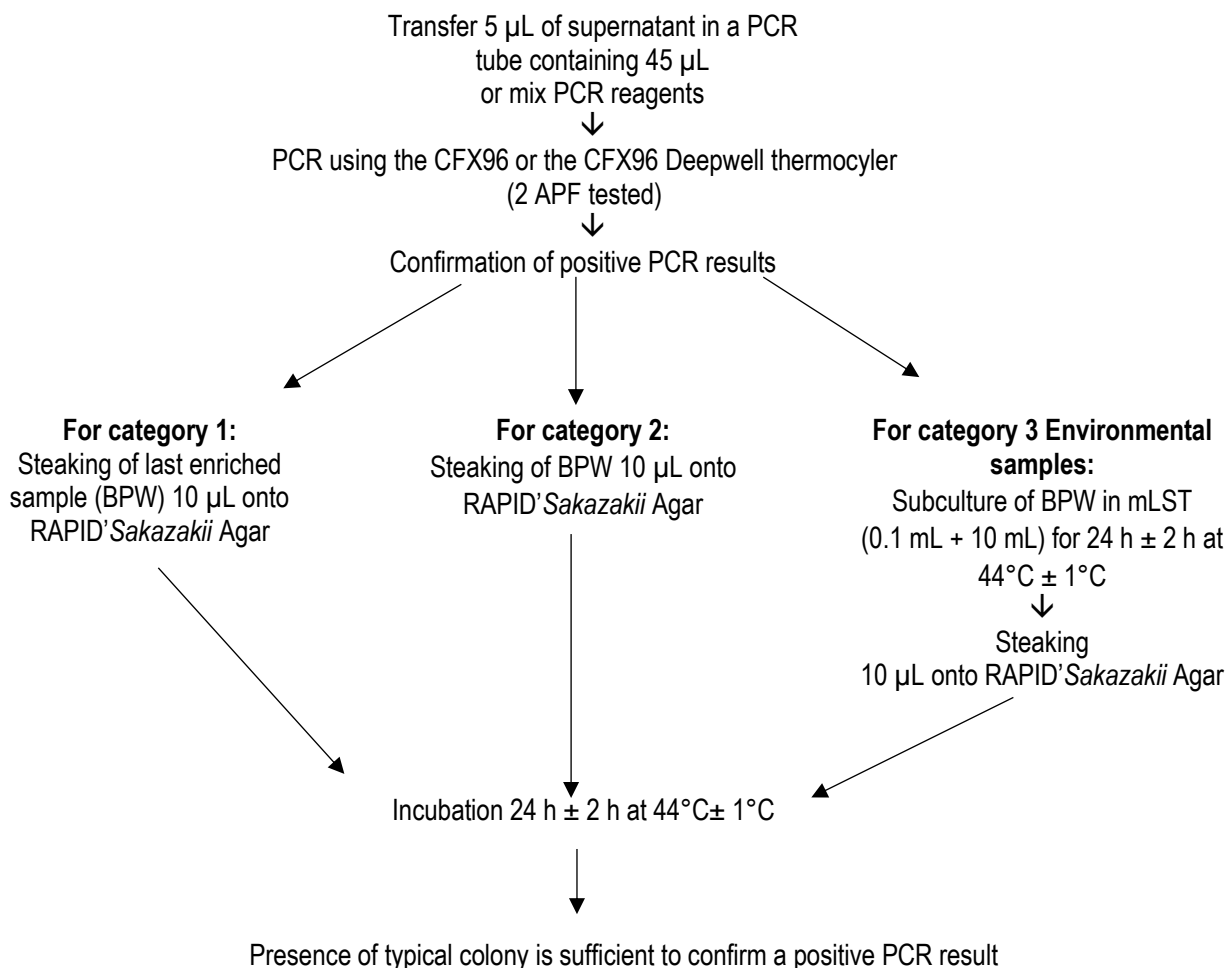
Appendix 1 – Flow diagram of the alternative method: iQ-Check *Cronobacter* spp.



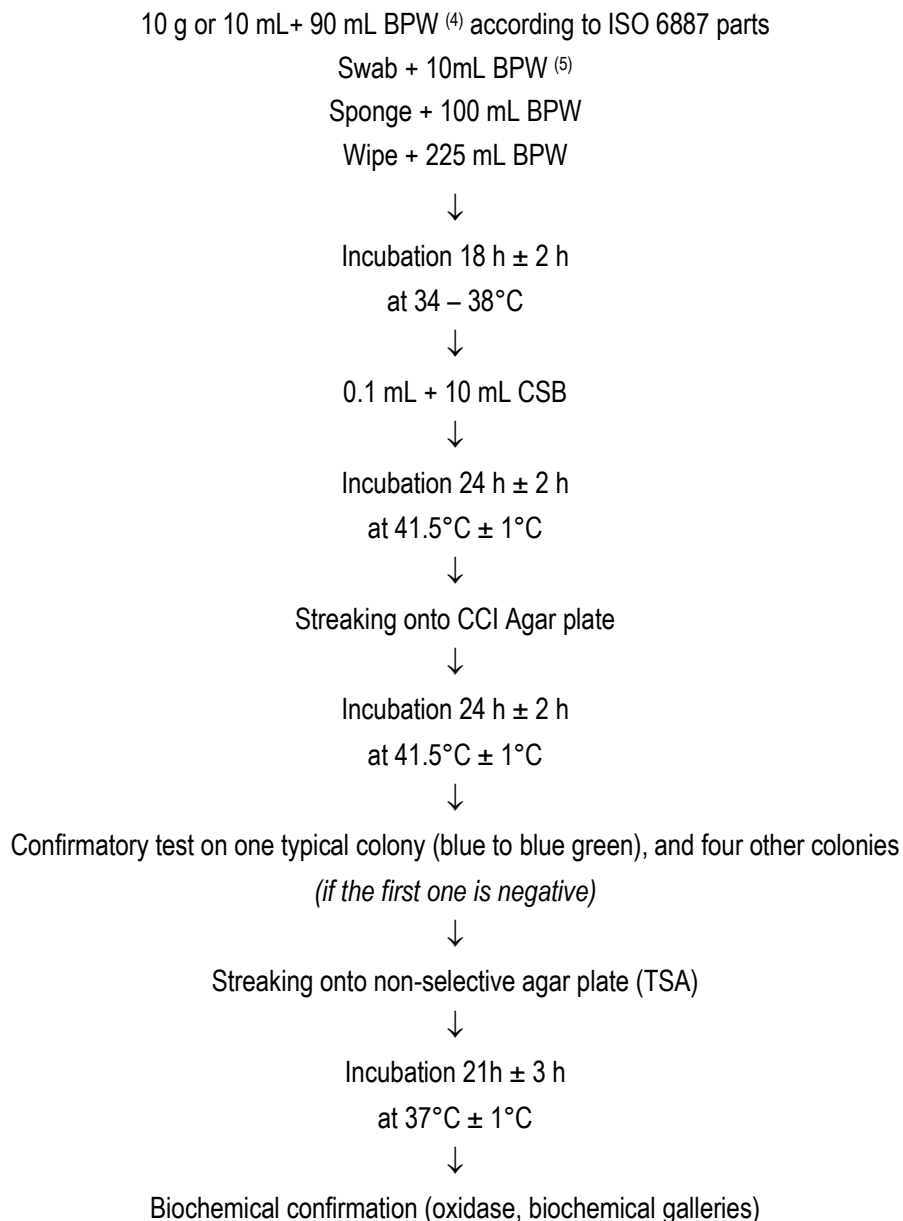
* Addition of α -amylase for infant cereals (0.1 g/L)

- 2 For sampling after cleaning process pre-moisten
 - 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)

3 During the validation study, a subculture in CSB broth for 24 h ± 2 h at 41.5°C was performed before streaking onto CCI Agar (24 h ± 2 h at 41.5°C ± 1°C) for negative PCR samples



**Appendix 2 - Flow diagram of the reference method:
ISO 22964 (April 2017) - Microbiology of the food chain -
Horizontal method for the detection of *Cronobacter* spp.**



⁴ Addition of α -amylase for infant cereals (0.1 g/L)

⁵ 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 3 – Artificial contamination of samples

Q-Lab
ADRIA

Year of analysis	Sample N°	Product	Artificial contaminations					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measurement	Inoculation level/sample CFU/sample			
2020	6a	Multigrain banana infant cereal	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	2	+	1	a
2020	9a	Oatmeal banana infant cereal	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.2	+	1	a
2020	10a	single grain raisin infant cereal	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.2	+	1	a
2020	12a	Pure Bliss Infant Formula	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.2	+	1	a
2020	14a	Infant formula with iron, 0-12 Months	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.2	+	1	a
2020	20a	Organic Stage 2 infant formula	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	3.8	+	1	a
2020	2b	Rice and banana apple - infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	3.8	+	1	b
2020	3b	Multi-Grain - infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	3.8	+	1	b
2020	6b	Oats and Quinoa - infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	3.8	+	1	b
2020	8b	Oatmeal Infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	3.8	+	1	b
2020	13b	Good Start - infant formula with probiotics	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.8	+	1	b
2020	18b	Sensitive - infant formula with probiotics	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.8	+	1	b
2020	19b	Fat malabsorption problems - infant formula with probiotics	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.8	+	1	b

Year of analysis	Sample N°	Product	Artificial contaminations					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measurement	Inoculation level/sample CFU/sample			
2020	1c	Organic Coconut oil	<i>Cronobacter sakazakii</i> FSL F6-044	Food	Heat treatment (50 - 55°C for 10-15 minutes)	0,56	3	+	1	c
2020	2c	Whole Wheat Flour	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.8	+	1	c
2020	5c	Old Fashioned Oats	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Stored for 2 weeks at room temperature (20-25°C)	/	4.8	+	1	c
2020	8c	Organic Soy Oil	<i>Cronobacter sakazakii</i> FSL F6-044	Food	Heat treatment (50 - 55°C for 10-15 minutes)	0,56	3	+	1	c
2020	11c	Non-fat dry milk powder	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Stored for 2 weeks at room temperature (20-25°C)	/	2.5	+	1	c
2020	13c	Instant milk powder	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Stored for 2 weeks at room temperature (20-25°C)	/	2.5	+	1	c
2020	14c	Skim milk powder	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Stored for 2 weeks at room temperature (20-25°C)	/	2.5	+	1	c
2020	16c	Whey protein	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Stored for 2 weeks at room temperature (20-25°C)	/	2.5	+	1	c
2020	17c	Organic sunflower oil	<i>Cronobacter sakazakii</i> FSL F6-044	Food	Heat treatment (50 - 55°C for 10-15 minutes)	0,56	3	+	1	c
2019	7720	Infant cereals without probiotic multi cereals	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.5	+	2	a
2019	7721	Infant cereals without probiotic brioche	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.5	+	2	a
2019	7722	Infant cereals without probiotic 5 cereals	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.5	+	2	a
2019	7723	Infant cereals without probiotic vanilla (10 months+)	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.5	+	2	a
2019	7724	Infant cereals without probiotic cocoa biscuit	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.5	+	2	a
2019	7725	Infant cereals without probiotic vanilla	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.5	+	2	a
2019	7726	Infant formula stage 2	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.3	+	2	a
2019	7727	Infant formula stage 2	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.3	+	2	a

Year of analysis	Sample N°	Product	Artificial contaminations					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measurement	Inoculation level/sample CFU/sample			
2019	7728	Infant formula stage 2 organic	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.3	+	2	a
2019	7729	Follow-up infant formula	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.3	+	2	a
2019	7730	Infant formula stage 1	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.3	+	2	a
2019	7731	Infant formula stage 2	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.3	+	2	a
2019	7732	Infant formula with probiotics stage 2 (<i>B.infantis</i> 8,3.10 ⁴ CFU/g)	<i>Cronobacter turincensis</i> Ad1445	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.4	+	2	b
2019	7733	Infant formula with probiotics thick formula with starch stage 2 (<i>Bifidobacteria</i> 8,3.10 ⁴ CFU/g)	<i>Cronobacter turincensis</i> Ad1445	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.4	+	2	b
2019	7734	Infant formula with probiotics thick formula stage 2 (<i>B.infantis</i> 3,7.10 ⁶ CFU/g)	<i>Cronobacter turincensis</i> Ad1445	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.4	+	2	b
2019	7735	Infant formula with probiotics stage 2 (<i>L. reuteri</i> 9,8.10 ⁵ CFU/g)	<i>Cronobacter sakazakii</i> Ad2349	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.9	+	2	b
2019	7736	Infant formula with probiotics thick formula stage 2 (<i>Bifidobacteria</i> 1,5.10 ³ CFU/g)	<i>Cronobacter sakazakii</i> Ad2349	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.9	+	2	b
2019	7737	Infant formula with probiotics stage 2 (<i>B.lactis</i> 5,2.10 ⁵ CFU/g)	<i>Cronobacter sakazakii</i> Ad2349	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2.9	-	2	b
2019	7738	Infant cereals with probiotics vanilla (<i>B. lactis</i> 5,3.10 ⁵ CFU/g)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.1	+	2	b
2019	7739	Infant cereals with probiotics 5 cereals (<i>B. lactis</i> 2,7.10 ⁶ CFU/g)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.1	+	2	b
2019	7740	Infant cereals with probiotics cocoa vanilla (<i>B. lactis</i> 4,5.10 ⁶ CFU/g)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.1	+	2	b
2019	7741	Infant cereals with probiotics biscuit (<i>B. lactis</i> 1,1.10 ⁶ CFU/g)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.1	+	2	b

Year of analysis	Sample N°	Product	Artificial contaminations					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measurement	Inoculation level/sample CFU/sample			
2019	7742	Infant cereals with probiotics oat and wheat (B. lactis 2,7.10 ⁶ CFU/g)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.1	+	2	b
2019	7743	Infant cereals with probiotics biscuit and nut (B. lactis 4,0.10 ⁶ CFU/g)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.1	+	2	b
2019	7744	Maltodextrin	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.0	+	2	c
2019	7745	Maltodextrin	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0.4	+	2	c
2019	7746	Non-fat dry milk powder	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.0	+	2	c
2019	7747	Non-fat dry milk powder	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0.4	+	2	c
2019	7748	Whey	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.0	-	2	c
2019	7749	Whey	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0.4	-	2	c
2019	7750	Whey	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0.4	+	2	c
2019	7751	Maltodextrin	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0.4	+	2	c
2019	7752	Whey	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.0	+	2	c
2019	7753	Non-fat dry milk powder	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0.4	+	2	c
2019	7754	Non-fat dry milk powder	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.0	+	2	c
2019	7755	Non-fat dry milk powder	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1.0	+	2	c
2020	2d	Process Water (Post clean - Port 1) - Spice Manufacturer	<i>Cronobacter dublinensis</i> CCUG 58095	Water fountain Basin	Heat treatment (50 - 55°C for 10-15 minutes)	0,8	1.8	+	3	a
2020	4d	Process Water (Post clean - Port 2)-Milk and Dairy Manufacturer	<i>Cronobacter dublinensis</i> CCUG 58095	Water fountain Basin	Heat treatment (50 - 55°C for 10-15 minutes)	0,8	1.8	+	3	a

Year of analysis	Sample N°	Product	Artificial contaminations					Global result	Category	Type
			Strain	Origin	Injury protocol	Injury measurement	Inoculation level/sample CFU/sample			
2020	7d	Process Water (Preclean - Port 4)-Spice Manufacturer	<i>Cronobacter dublinensis</i> CCUG 58095	Water fountain Basin	Heat treatment (50 - 55°C for 10-15 minutes)	0,8	1.8	+	3	a
2020	8d	Process Water (Post clean - Port 4)-Spice Manufacturer	<i>Cronobacter dublinensis</i> CCUG 58095	Water fountain Basin	Heat treatment (50 - 55°C for 10-15 minutes)	0,8	1.8	+	3	a
2020	9d	Process Water (Preclean - Port 5)-Milk and Dairy Manufacturer	<i>Cronobacter dublinensis</i> CCUG 58095	Water fountain Basin	Heat treatment (50 - 55°C for 10-15 minutes)	0,8	1.8	+	3	a
2020	12d	Process Water (Post clean - Hose 1)-Spice Manufacturer	<i>Cronobacter sakazakii</i> CCUG 64760	Environment, Industry	Heat treatment (50 - 55°C for 10-15 minutes)	0,84	4	+	3	a
2020	14d	Process Water (Post clean - Hose 2)-Spice Manufacturer	<i>Cronobacter sakazakii</i> CCUG 64760	Environment, Industry	Heat treatment (50 - 55°C for 10-15 minutes)	0,84	4	+	3	a
2020	15d	Process Water (Preclean - Hose 3)-Milk and Dairy Manufacturer	<i>Cronobacter sakazakii</i> CCUG 64760	Environment, Industry	Heat treatment (50 - 55°C for 10-15 minutes)	0,84	4	+	3	a
2020	21e	Swab - Doorhandle #6 - Milk and Dairy Manufacturer	<i>Cronobacter muytjensii</i> QL 17031.6	Environmental Isolate	48 hours at 4°C	/	1.8	+	3	b
2020	22e	Swab - Employee glove - post shift (Left) - Spice Manufacturer	<i>Cronobacter muytjensii</i> QL 17031.6	Environmental Isolate	48 hours at 4°C	/	1.8	+	3	b
2020	23e	Sponge - Mixing Blade #8 (Preclean) - Milk and Dairy Manufacturer	<i>Cronobacter muytjensii</i> QL 17031.6	Environmental Isolate	48 hours at 4°C	/	1.8	+	3	b
2020	24e	Swab - Employee glove - post shift (Right) - Spice Manufacturer	<i>Cronobacter muytjensii</i> QL 17031.6	Environmental Isolate	48 hours at 4°C	/	1.8	+	3	b
2020	25e	Sponge - Employee shoe - post shift (Left) - Spice Manufacturer	<i>Cronobacter muytjensii</i> QL 17031.6	Environmental Isolate	48 hours at 4°C	/	1.8	+	3	b
2020	26e	Sponge - Employee shoe - post shift (Right) - Spice Manufacturer	<i>Cronobacter sakazakii</i> CCUG 28870	Dish Brush	48 hours at 4°C	/	2.25	+	3	b
2020	27e	Swab - Drain #4 (Preclean) - Milk and Dairy Manufacturer	<i>Cronobacter sakazakii</i> CCUG 28870	Dish Brush	48 hours at 4°C	/	2.25	+	3	b
2020	28e	Sponge - Trash Can #4 - Milk and Dairy Manufacturer	<i>Cronobacter sakazakii</i> CCUG 28870	Dish Brush	48 hours at 4°C	/	2.25	+	3	b
2020	29e	Sponge - Conveyer belt #3 (Preclean) - Milk and Dairy Manufacturer	<i>Cronobacter sakazakii</i> CCUG 28870	Dish Brush	48 hours at 4°C	/	2.25	+	3	b

Appendix 4 – Sensitivity study: raw data

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964				Alternative method: iQ -Check Cronobacter spp																								Category	Type						
		30 g + 270 mL BPW + 10 µg /mL vancomycin - 20h ± 2h at 37°C ± 1°C + 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C																																			
		PCR												RAPID'Sakazakii (direct streaking 10 µl)												Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol					
		Easy protocol												Confirmation														APF				APF with FDRS		APF Fast		APF Fast with FDRS	
		APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Without purification step		With purification step		APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast			APF Fast with FDRS									
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq		Final result	Oxidase	Biochemical gallery (API ID32)	Oxidase								Biochemical gallery (API ID32)											
1a	Organic baby cereal	+1/2	-	Cronobacter spp.	+	20	32.83	+	20	32.72	+	20	33.88	+	20	33.39	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
2a	Step 1 infant cereal	-	/	/	-	-	32.76	-	-	32.68	-	-	33.6	-	-	33.26	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
3a	Step 3 infant cereal	-	/	/	-	-	32.55	-	-	32.39	-	-	33.46	-	-	33.08	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
4a	Stage 1 infant cereal	-	/	/	-	-	33.05	-	-	32.77	-	-	33.75	-	-	33.35	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
5a	Stage 2 infant cereal	+1/2	-	Cronobacter spp.	+	28.7	-	+	23	33.17	+	20	34.38	+	20	33.24	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
6a	Multigrain banana infant cereal	+1/2	-	Cronobacter spp.	+	17.9	33.66	+	19.35	34.04	+	19	33.80	+	20	33.12	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
7a	oatmeal	-	/	/	-	-	36.29	-	-	36	-	-	33.34	-	-	33.31	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
8a	single grain infant cereal	-	/	/	-	-	35.51	-	-	35.83	-	-	33.49	-	-	33.44	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
9a	Oatmeal banana infant cereal	+1/2	-	Cronobacter spp.	+	21.9	32.72	+	23	33.25	+	22	33.38	+	22	32.98	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
10a	single grain raisin infant cereal	+1/2	-	Cronobacter spp.	+	20	33.56	+	39	-	+	20	33.83	+	20	33.25	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
11a	Neosure infant formula	-	/	/	-	-	34.61	-	-	36.36	-	-	33.45	-	-	33.5	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
12a	Pure Bliss Infant Formula	+1/2	-	Cronobacter spp.	+	20.6	32.86	+	26	37.72	+	20	33.63	+	20	33.53	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
13a	State 1 Non-GMO Infant Formula	+1/2	-	Cronobacter spp.	+	19.5	32.9	+	20	32.96	+	20	64.08	+	20	33.61	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
14a	Infant formula with iron, 0-12 Months	+1/2	-	Cronobacter spp.	+	21.5	32.09	+	22	32.48	+	22	33.37	+	22	33.48	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
15a	Stage 23 infant formula	-	/	/	-	-	35.56	-	-	32.85	-	-	33.36	-	-	33.51	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
16a	Soy based infant formula	-	/	/	-	-	36.16	-	-	32.94	-	-	33.55	-	-	33.55	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
17a	Non-GMO Infant Formula	+1/2	-	Cronobacter spp.	+	22.1	32.25	+	23	32.05	+	22	33.11	+	22	33.52	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
18a	Infant formula with iron	+1/2	-	Cronobacter spp.	+	19.3	32.93	+	20	32.62	+	20	34.55	+	20	33.94	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			
19a	Organic infant formula with lactose	-	/	/	-	-	33.04	-	-	32.85	-	-	33.51	-	-	33.38	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	a				
20a	Organic Stage 2 infant formula	+1/2	-	Cronobacter spp.	+	19.7	33.11	+	20	32.43	+	20	33.53	+	21	33.72	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	a			

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ -Check <i>Cronobacter</i> spp																								Category	Type							
				30 g + 270 mL BPW + 10 µg /mL vancomycin - 20h ± 2h at 37°C ± 1°C + 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C																																
		CCI	Oxidase	Identification	Result	PCR												RAPID'Sakazakii (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol						
						Easy protocol						Confirmation						Without purification step		With purification step																
						APF		APF with FDRS		APF Fast		APF Fast with FDRS		Typical colonies	Without purification step		With purification step		APF		APF with FDRS			APF Fast		APF Fast with FDRS										
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)		Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS												
1b	Frawler - infant cereal with probiotics (8.2.10 ⁶ CFU/g)	-	/	/	-	-	32.34	-	-	32.26	-	-	33.14	-	-	31.41	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	b
2b	Rice and banana apple - infant cereal with probiotics (4.9.10 ⁶ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	20.4	41.87	+	19	33.35	+	21	36.75	+	19	36.8	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
3b	Multi-Grain - infant cereal with probiotics (6.5.10 ⁶ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	20	32.16	+	19	30.99	+	20	33.16	+	20	32.16	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
4b	Organic with DHA - Infant Cereal with probiotics (2.3.10 ⁶ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	19.9	35.93	+	19	32.29	+	20	36.8	+	19	33.23	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
5b	Single grain rice - infant cereal with probiotics (5.0.10 ⁶ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	18.9	35.28	+	19	33.37	+	19	35.39	+	19	33.14	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
6b	Oats and Quinoa - infant cereal with probiotics (8.9.10 ⁵ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	20.3	32.71	+	20	31.17	+	21	33.49	+	21	32.11	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
7b	Single grain variety pack - infant cereal with probiotics (8.2.10 ⁶ CFU/g)	-	/	/	-	-	32.46	-	-	31.64	-	-	33.35	-	-	32.65	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	b			
8b	Oatmeal Infant cereal with probiotics (1.3.10 ⁶ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	19.2	35.95	+	20	35.03	+	20	36.94	+	20	33.57	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
9b	Organic oatmeal with choline - infant cereal with probiotics (1.8.10 ⁶ CFU/g)	-	/	/	-	-	32.67	-	-	32.66	-	-	33.17	-	-	32.83	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	b			
10b	Oatmeal banana strawberry - infant cereal with probiotics (4.5.10 ⁶ CFU/g)	+1/2	-	<i>Cronobacter</i> spp.	+	20.4	34.61	+	19	35.99	+	20	36.68	+	20	34.81	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ -Check Cronobacter spp																								Category	Type							
				30 g + 270 mL BPW + 10 µg /mL vancomycin - 20h ± 2h at 37°C ± 1°C + 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C																																
		CCI	Oxidase	Identification	Result	PCR												RAPID'Sakazakii (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol						
						Easy protocol												Confirmation																		
						APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Without purification step		With purification step															
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS		APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS												
11b	Comfort - infant formula with probiotics (9.6.10 ⁵ CFU/g)	-	/	/	-	-	32.78	-	-	32.75	-	-	33.17	-	-	32.77	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	b
12b	With iron - infant formula with probiotics (7.2.10 ⁶ CFU/g)	-	/	/	-	-	32.35	-	-	32.44	-	-	32.91	-	-	33.09	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	b
13b	Good Start - infant formula with probiotics (6.8.10 ⁶ CFU/g)	+1/2	-	Cronobacter spp.	+	19.3	36.64	+	20	35.25	+	19	38.94	+	20	35.39	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
14b	For spit up - infant formula with probiotics (8.810 ⁶ CFU/g)	-	/	/	-	-	32.34	-	-	32.75	-	-	32.85	-	-	32.95	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	b
15b	Total comfort - infant formula with probiotics (2.9.10 ⁶ CFU/g)	-	/	/	-	-	32.44	-	-	32.85	-	-	33.15	-	-	33.27	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	b
16b	Organic Lactose - infant formula with probiotics (5.7.10 ⁶ CFU/g)	+1/2	-	Cronobacter spp.	+	18.3	37.33	+	19	36.77	+	19	39.46	+	20	35.52	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
17b	Milk based - infant formula with probiotics (3.6.10 ⁶ CFU/g)	+1/2	-	Cronobacter spp.	+	21	38.34	+	19	35.87	+	20	39.39	+	20	35.54	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
18b	Sensitive - infant formula with probiotics (7.8.10 ⁵ CFU/g)	+1/2	-	Cronobacter spp.	+	21.2	32.58	+	20	32.07	+	20	33.28	+	20	32.39	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
19b	Fat malabsorption problems - infant formula with probiotics (3.1.10 ⁶ CFU/g)	+1/2	-	Cronobacter spp.	+	19.8	36.76	+	20	34.41	+	19	38.42	+	20	35.32	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	b		
20b	Gentle start - infant formula with probiotics (6.4.10 ⁶ CFU/g)	-	/	/	-	-	33	-	-	36.47	-	-	33.41	-	-	33.54	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	b
1c	Organic Coconut oil	+1/2	-	Cronobacter spp.	+	21.6	33.84	+	20	33.04	+	23	33.02	+	21	31.32	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c		
2c	Whole Wheat Flour	+1/2	-	Cronobacter spp.	+	29.7	32.14	+	30	32.04	+	31	32.41	+	29	30.79	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c		
3c	Organic Brown Rice Flour	+1/2	-	Cronobacter spp.	+	35.37/ 18.5	33.39/ 31.57	+/+	34.76/ 18.7	32.99/ 31.73	+/+	36.47/ 23.06	33.02/ 33.8	+/+	34.3/ 30.47	31.74/ 36.51	+/+	d (- on several plates)	-	Enterobacter cloacae	-	Enterobacter cloacae	-	-	-	-	-	-	PA FP(alt)	PA FP(alt)	PA FP(alt)	PA FP(alt)	1	c		
4c	Quinoa Flour	-	/	/	-	-	32.32	-	-	32.15	-	-	32.43	-	-	31.75	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	1	c

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ -Check Cronobacter spp																								Category	Type					
				30 g + 270 mL BPW + 10 µg /mL vancomycin - 20h ± 2h at 37°C ± 1°C + 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C																														
		CCI	Oxidase	Identification	Result	PCR												RAPID'Sakazakii (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol				
						Easy protocol						Confirmation						Without purification step		With purification step														
						APF		APF with FDRS		APF Fast		APF Fast with FDRS		Typical colonies					APF	APF with FDRS	APF Fast			APF Fast with FDRS	APF	APF with FDRS	APF Fast			APF Fast with FDRS				
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)		Oxidase	Biochemical gallery (API ID32)																		
5c	Old Fashioned Oats	+1/2	-	Cronobacter spp.	+	25.5	33.28	+	25	32.35	+	26	32.64	+	25	31.13	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
6c	Brown Rice Flour	+1/2	-	Cronobacter spp.	+	21.4	34.09	+	21	33.17	+	23	32.98	+	21	31.88	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
7c	All purpose Flour	+1/2	-	Cronobacter spp.	+	21.5	33.97	+	21	33.35	+	23	33.54	+	21	32.18	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
8c	Organic Soy Oil	+1/2	-	Cronobacter spp.	+	20.3	34.63	+	19	33.57	+	21	34.19	+	20	32.81	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
9c	Sodium Citrate	-	/	/	-	-	32.84	-	-	33.39	-	-	33.08	-	-	32.78	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	c	
10c	Folic Acid	-	/	/	-	-	32.99	-	-	32.98	-	-	32.88	-	-	32.51	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	c	
11c	Nonfat dry milk powder	+1/2	-	Cronobacter spp.	+	21.5	33.62	+	21	34.13	+	23	33.03	+	21	32.74	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
12c	Organic goat milk powder	-	/	/	-	-	32.93	-	-	32.87	-	-	32.2	-	-	32.27	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	c	
13c	Instant milk powder	+1/2	-	Cronobacter spp.	+	25.3	33.25	+	25	31.77	+	26	32.4	+	26	31.42	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
14c	Skim milk powder	+1/2	-	Cronobacter spp.	+	29.6	32.22	+	29	32.08	+	31	33.04/33.01	+	30	31.76	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
15c	Organic fat free milk powder	+1/2	-	Cronobacter spp.	+	35.26/18.47	33.26/32.12	+/-	34.61/18.61	33.02/31.82	+/-	36.91/22.79	34.04/34.18	+/-	35.37/29.19	32.52/34.52	+/-	d (- on several plates)	-	Enterobacter cloacae	-	Enterobacter cloacae	-	-	-	-	-	-	PA FP(alt)	PA FP(alt)	PA FP(alt)	PA FP(alt)	1	c
16c	Whey protein	+1/2	-	Cronobacter spp.	+	21.6	34.1	+	21	32.74	+	23	32.57	+	22	32.23	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
17c	Organic sunflower oil	+1/2	-	Cronobacter spp.	+	21.5	34.38	+	21	34.3	+	23	34.16	+	22	32.81	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
18c	Manganese sulfate	-	/	/	-	-	32.32	-	-	32.38	-	-	32.57	-	-	32.24	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	c	
19c	Sinc sulfate	+1/2	-	Cronobacter spp.	+	20.3	35.34	+	19	35.28	+	22	34.16	+	20	33.38	+	+M	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	1	c
20c	Biotin	-	/	/	-	-	33.19	-	-	33.21	-	-	32.75	-	-	32.63	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	1	c	

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) (Study realized by Q-Laboratories Inc.)																																
Sample N°	Product	ISO 22964	Alternative method: iQ-Check <i>Cronobacter</i> spp																									Category	Type			
			30 g + 270 mL BPW + 10 µg/mL vancomycin - 20h ± 2h at 37°C ± 1°C + 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C+ storage 48h enrichment																													
			PCR - 48h enrichment												RAPID [®] <i>sakazakii</i> (direct streaking 10 µl) - 48h enrichment				All confirmatory tests				Final Result Easy protocol -48h enrichment				Agreement Easy Protocol - 48h enrichment					
			Easy protocol												Confirmation																	
			APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Without purification step		APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS							
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	target Cq	I.C. Cq	Final result	Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)																			
1a	Organic baby cereal	+	21	32.15	+	NA/29.64*	NA/32.81*	i/+*	22	33.07	+	23	32.78	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
5a	Stage 2 infant cereal	+	21	32.23	+	31.92	37.02	+	22	32.9	+	22	33.06	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
6a	Multigrain banana infant cereal	+	21	33.80	+	21.31	32.67	+	21	37.22	+	23	-	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
9a	Oatmeal banana infant cereal	+	22	31.96	+	24.37	31.64	+	23	33.06	+	24	32.56	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
10a	single grain raisin infant cereal	+	21	33.00	+	21.47	32.66	+	22	33.51	+	23	33.01	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
12a	Pure Bliss Infant Formula	+	21	32.5	+	21.59	32.25	+	22	33.16	+	22	32.64	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
13a	State 1 Non-GMO Infant Formula	+	21	32.01	+	21.59	32.21	+	22	33.24	+	22	32.86	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
14a	Infant formula with iron, 0-12 Months	+	24	31.49	+	25.52	31.58	+	25	32.55	+	26	32.4	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
17a	Non-GMO Infant Formula	+	22	32.16	+	22.36	32.11	+	23	32.82	+	23	32.42	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
18a	Infant formula with iron	+	21	32.25	+	22.00	32.34	+	22	32.93	+	23	32.86	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
20a	Organic Stage 2 infant formula	+	21	20.83	+	21.85	32.2	+	22	33.02	+	23	32.79	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	a				
2b	Rice and banana apple - infant cereal with probiotics	+	21	34.25	+	22.50	32.93	+	22	34.41	+	21	31.37	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
3b	Multi-Grain - infant cereal with probiotics	+	21	32.2	+	22.82	32.08	+	22	32.45	+	21	31.31	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
4b	Organic with DHA - Infant Cereal with probiotics	+	20	34.49	+	22.2	33.19	+	21	35.28	+	21	32.15	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
5b	Single grain rice - infant cereal with probiotics	+	21	33.04	+	22.07	32.63	+	22	33.6	+	22	32.35	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
6b	Oats and Quinoa - infant cereal with probiotics	+	24	33.04	+	26.58	32.19	+	26	32.35	+	27	31.76	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
8b	Oatmeal Infant cereal with probiotics	+	21	34.40	+	21.59	33.07	+	22	34.69	+	22	33.75	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
10b	Oatmeal banana strawberry - infant cereal with probiotics	+	20	35.01	+	21.15	33.48	+	21	35.68	+	22	34.03	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
13b	Good Start - infant formula with probiotics	+	19	35.28	+	22.72	32.69	+	21	36.39	+	24	32.92	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
16b	Organic Lactose - infant formula with probiotics	+	19	36.5	+	23.52	36.55	+	21	38.2	+	21	35.61	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
17b	Milk based - infant formula with probiotics	+	21	33.95	+	25.08	33.93	+	22	34.99	+	23	34.13	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
18b	Sensitive - infant formula with probiotics	+	22	31.77	+	33.71	34.22	+	23	32.79	+	26	32.28	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
19b	Fat malabsorption problems - infant formula with probiotics	+	21	33.50	+	NA/30.11*	NA/32.62*	i/+*	22	35.03	+	24	33.87	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	b				
1c	Organic Coconut oil	+	22	33.69	+	20.32	30.82	+	22	33.15	+	21	32.78	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				
2c	Whole Wheat Flour	+	31	32.07	+	29.71	31.09	+	31	32.23	+	31	32.07	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				
3c	Organic Brown Rice Flour	+	35	32.28	+	34.04	31.88	+	35	32.47	+	36	32.48	+	d (- on several plates)	-	<i>Enterobacter cloacae</i>	-	-	-	-	-	PA FP(alt)	PA FP(alt)	PA FP(alt)	PA FP(alt)	1	c				
5c	Old Fashioned Oats	+	26	32.46	+	25.22	31.42	+	26	32.29	+	26	32.09	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				
6c	Brown Rice Flour	+	22	33.2	+	21.22	32.66	+	22	32.99	+	22	32.44	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				
7c	All purpose Flour	+	22	33.54	+	21.54	32.96	+	22	33.18	+	22	32.55	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				
8c	Organic Soy Oil	+	21	34.30	+	20.65	33.84	+	20	33.42	+	20	32.6	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				
11c	Non-fat dry milk powder	+	22	33.83	+	21.22	32.76	+	22	32.78	+	21	32.64	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c				

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) (Study realized by Q-Laboratories Inc.)																													
Sample N°	Product	ISO 22964	Alternative method: iQ-Check <i>Cronobacter</i> spp																									Category	Type
			30 g + 270 mL BPW + 10 µg /mL vancomycin - 20h ± 2h at 37°C ± 1°C + 1mL enriched BPW + 9mL BPW 4h± 1h at 37°C ± 1°C+ storage 48h enrichment																										
			PCR - 48h enrichment												RAPID [®] sakazakii (direct streaking 10 µl) - 48h enrichment				All confirmatory tests	Final Result Easy protocol -48h enrichment				Agreement Easy Protocol - 48h enrichment					
			Easy protocol												Confirmation					APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS		
			APF			APF with FDRS			APF Fast			APF Fast with FDRS			Without purification step		Typical colonies												
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	target Cq	I.C. Cq	Final result	Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast		APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS							
13c	Instant milk powder	+	26	32.48	+	26.37	32.1	+	26	32.28	+	26	31.88	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c	
14c	Skim milk powder	+	31	32.26	+	30.97	32.08	+	30	31.55	+	31	31.78	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c	
15c	Organic fat free milk powder	+	35	32.64	+	35.07	32.73	+	35	32.59	+	35	32.61	+	d (- on several plates)	-	<i>Enterobacter cloacae</i>	-	-	-	-	-	PA FP(alt)	PA FP(alt)	PA FP(alt)	PA FP(alt)	1	c	
16c	Whey protein	+	22	33.56	+	21.71	33.96	+	22	32.99	+	22	32.76	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c	
17c	Organic sunflower oil	+	22	33.80	+	21.44	33.16	+	22	33.02	+	22	32.55	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c	
19c	Sinc sulfate	+	21	34.88	+	20.68	33..54	+	20	33.29	+	20	32.59	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	1	c	

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																																		
Sample N°	Product	Product (French name)	ISO 22964 [♦]				Alternative method: iQ-Check <i>Cronobacter</i> spp																											
			CCI	Oxidase	Identification	Result	375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C																											
							PCR												RAPID [®] <i>Sakazakii</i> (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol					
							Easy protocol												Confirmation															
							APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Without purification step		With purification step												
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast		APF Fast with FDRS	APF	APF with FDRS			APF Fast	APF Fast with FDRS								
8012	Infant formula stage 2 organic	Poudre de lait infantile bio 2ème âge sans probiotique	st	/	/	-	-	33.06	-	-	32.77	-	-	32.96	-	-	33.02	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8013	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	st	/	/	-	-	32.25	-	-	32.88	-	-	33.28	-	-	33.08	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8014	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	st	/	/	-	-	33.10	-	-	32.79	-	-	32.83	-	-	32.66	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8015	Follow-up infant formula	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	st	/	/	-	-	32.83	-	-	32.85	-	-	32.94	-	-	32.60	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8016	Infant cereals without probiotic vanilla (10 months+)	Céréales infantiles sans probiotique babivanille (10+ mois)	st	/	/	-	-	33.92	-	-	33.44	-	-	33.71	-	-	33.68	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8017	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	st	/	/	-	-	34.39	-	-	33.38	-	-	33.87	-	-	33.14	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8018	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	st	/	/	-	-	33.73	-	-	33.52	-	-	33.88	-	-	33.15	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	a
8019	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	st	/	/	-	31.46	33.01	+	31.08	32.60	+	30.11	32.08	+	30.24	33.19	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PD	PD	PD	PD	2	a
8020	Infant cereals without probiotic 5 cereals	Céréales infantiles sans probiotique 5 céréales	st	/	/	-	-	34.80	-	-	34.74	-	-	34.66	-	-	33.00	-	-	/	/	/	/	-	-	-	-	NA	NA	NA	NA	2	a	
7732	Infant formula with probiotics stage 2 (B.infantis 8,3.10 ⁴ CFU/g)	Poudre de lait avec probiotiques 2ème âge (B.infantis 8,3.10 ⁴ CFU/g)	+p	-	+	+	21.81	40.12	+	21.66	34.15	+	21.33	42.97	+	22.56	37.96	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7733	Infant formula with probiotics thick formula with starch stage 2 (Bifidobacteria 8,3.10 ⁴ CFU/g)	Poudre de lait avec probiotiques formule épaissie amidon 2ème âge (Bifidobacteries 8,3.10 ⁴ CFU/g)	st	/	/	-	23.98	38.76	+	24.04	38.16	+	24.87	35.42	+	25.52	33.61	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PD	PD	PD	PD	2	b
7734	Infant formula with probiotics thick formula stage 2 (B.infantis 3,7.10 ⁶ CFU/g)	Poudre de lait avec probiotiques actigest formule épaisse 2ème âge (B.infantis 8,3.10 ⁴ CFU/g)	+p	-	+	+	19.53	34.50	+	20.42	32.80	+	21.19	35.94	+	20.00	34.31	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																																		
Sample N°	Product	Product (French name)	ISO 22964 [♦]				Alternative method: iQ-Check <i>Cronobacter</i> spp																											
			CCI	Oxidase	Identification	Result	375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C																											
							PCR												RAPID [®] <i>Sakazakii</i> (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol					
							Easy protocol						Confirmation						Typical colonies	Without purification step		With purification step												
							APF		APF with FDRS		APF Fast		APF Fast with FDRS		Oxidase	Biochemical gallery (API ID32)	Oxidase	Biochemical gallery (API ID32)		APF	APF with FDRS	APF Fast			APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS					
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result																							
7735	Infant formula with probiotics stage 2 (L. reuteri 9,8.10 ⁵ CFU/g)	Poudre de lait avec probiotiques relais 2ème âge (L. reuteri 9,8.10 ⁵ CFU/g)	st	/	/	-	27.32	-	+	24.14	-	+	27.69	-	+	25.29	-	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PD	PD	PD	PD	2	b
7736	Infant formula with probiotics thick formula stage 2 (Bifidobacteria 1,5.10 ³ CFU/g)	Poudre de lait avec probiotiques formule épaissie 2ème âge (Bifidobactéries 1,5.10 ³ CFU/g)	+p	-	+	+	25.85	34.37	+	25.85	33.00	+	25.59	39.32	+	26.00	33.52	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7737	Infant formula with probiotics stage 2 (B.lactis 5,2.10 ⁵ CFU/g)	Poudre de lait avec probiotiques 2ème âge (B.lactis 5,2.10 ⁵ CFU/g)	st	/	/	-	-	32.84	-	-	33.11	-	-	32.71	-	-	32.58	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	2	b
7738	Infant cereals with probiotics vanilla (B. lactis 5,3.10 ⁵ CFU/g)	Céréales infantiles vanille avec probiotiques (B.lactis 5,3.10 ⁵ CFU/g)	+p	-	+	+	32.71	34.68	+	33.41	34.24	+	32.73	33.87	+	33.41	33.8	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7739	Infant cereals with probiotics 5 cereals (B. lactis 2,7.10 ⁶ CFU/g)	Céréales infantiles 5 céréales avec probiotiques (B.lactis 2,7.10 ⁶ CFU/g)	+p	-	+	+	30.63	34.8	+	34.87	34.41	+	29.91	33.68	+	33.11	33.06	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7740	Infant cereals with probiotics cocoa vanilla (B. lactis 4,5.10 ⁶ CFU/g)	Céréales infantiles vanille chocolat au lait avec probiotiques (B.lactis 4,5.10 ⁶ CFU/g)	+p	-	+	+	26.24	-	+	25.67	-	+	25.81	36.52	+	23.83	36.40	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7741	Infant cereals with probiotics biscuit (B. lactis 1,1.10 ⁶ CFU/g)	Céréales infantiles saveur biscuit avec probiotiques (B.lactis 1,1.10 ⁶ CFU/g)	+p	-	+	+	29.87	34.89	+	27.73	33.81	+	28.96	33.58	+	28.03	32.25	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7742	Infant cereals with probiotics oat and wheat (B. lactis 2,7.10 ⁶ CFU/g)	Céréales infantiles avoine et blé avec probiotiques (B.lactis 2,7.10 ⁶ CFU/g)	+p	-	+	+	26.55	35.12	+	29.9	-	+	28.39	41.04	+	27.47	34.62	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b
7743	Infant cereals with probiotics biscuit and nut (B. lactis 4,0.10 ⁶ CFU/g)	Céréales infantiles noisette biscuit avec probiotiques (B.lactis 4,0.10 ⁶ CFU/g)	+p	-	+	+	29.3	35.62	+	26.64	34.17	+	27.84	33.45	+	26.77	33.61	+	+p	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	PA	PA	PA	PA	2	b

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																																		
Sample N°	Product	Product (French name)	ISO 22964 [♦]				Alternative method: iQ-Check <i>Cronobacter</i> spp																				Category	Type						
			375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C																															
			PCR												RAPID'Sakazakii (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol									
			Easy protocol												Confirmation																			
			APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Without purification step		With purification step			APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS			APF Fast	APF Fast with FDRS				
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	Oxidase		Biochemical gallery (API ID32)																		
8104	Infant cereals with probiotics biscuit (B. lactis 1,1.10 ⁶ CFU/g)	Céréales infantiles saveur biscuit avec probiotiques (B.lactis 1,1.10 ⁶ CFU/g)	-	/	/	-	-	34.87	-	-	34.38	-	-	34.15	-	-	34.44	-	-	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8105	Infant cereals with probiotics 5 cereals (B. lactis 2,7.10 ⁶ CFU/g)	Céréales infantiles 5 céréales avec probiotiques (B.lactis 2,7.10 ⁶ CFU/g)	-	/	/	-	-	34.80	-	-	34.88	-	-	34.33	-	-	34.59	-	-	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8106	Infant cereals with probiotics cocoa vanilla (B. lactis 4,5.10 ⁶ CFU/g)	Céréales infantiles vanille chocolat au lait avec probiotiques (B.lactis 4,5.10 ⁶ CFU/g)	-	/	/	-	-	35.76	-	-	34.20	-	-	34.45	-	-	34.66	-	-	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8107	Infant cereals with probiotics biscuit and nut (B. lactis 4,0.10 ⁶ CFU/g)	Céréales infantiles noisette biscuit avec probiotiques (B.lactis 4,0.10 ⁶ CFU/g)	-	/	/	-	-	34.69	-	-	34.83	-	-	34.12	-	-	34.44	-	-	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8108	Infant cereals with probiotics vanilla (B. lactis 5,3.10 ⁵ CFU/g)	Céréales infantiles vanille avec probiotiques (B.lactis 5,3.10 ⁵ CFU/g)	-	/	/	-	-	35.76	-	-	34.85	-	-	34.57	-	-	34.58	-	-	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8109	Infant formula with probiotics thick formula stage 2 (Bifidobacteria 1,5.10 ⁶ CFU/g)	Poudre de lait avec probiotiques formule épaisse 2ème âge (Bifidobactéries 1,5.10 ⁶ CFU/g)	st	/	/	-	-	34.62	-	-	34.09	-	-	33.60	-	-	33.36	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8110	Infant formula with probiotics stage 2 (L. reuteri 3,2.10 ⁶ CFU/g)	Poudre de lait avec probiotiques relais 2ème âge (L. reuteri 3,2.10 ⁶ CFU/g)	st	/	/	-	-	34.82	-	-	34.14	-	-	33.88	-	-	34.21	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8111	Infant formula with probiotics stage 2 (Lactobacillus fermentum hereditum 1,4.10 ⁵ CFU/g)	Poudre de lait avec probiotiques 2ème âge (Lactobacillus fermentum hereditum 1,4.10 ⁵ CFU/g)	st	/	/	-	-	33.47	-	-	35.02	-	-	33.57	-	-	33.96	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b
8112	Infant formula with probiotics stage 2 (L. reuteri 6,5.10 ³ CFU/g)	Poudre de lait avec probiotiques relais 2ème âge (L. reuteri 6,5.10 ³ CFU/g)	st	/	/	-	-	33.44	-	-	34.24	-	-	34.09	-	-	34.00	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																																							
Sample N°	Product	Product (French name)	ISO 22964*				Alternative method: iQ-Check Cronobacter spp																																
			375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C																				Category	Type															
			PCR												RAPID'Sakazakii (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol														
			Easy protocol												Confirmation						Without purification step				With purification step														
			APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Without purification step		With purification step			APF	APF with FDRS			APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS									
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	Oxidase		Biochemical gallery (API ID32)																							
8113	Infant formula with probiotics stage 2 (Bifidobacteria 3.2.10 ⁴ CFU/g)	Poudre de lait avec probiotiques 2ème âge (Bifidobactéries 3.2.10 ⁴ CFU/g)	st	/	/	-	-	33.75	-	-	34.29	-	-	34.46	-	-	34.47	-			st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	b			
7744	Maltodextrin	Maltodextrine	st	/	/	-	21.26	34.21	+	21.4	34.54	+	22.21	32.72	+	21.51	32.68	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PD	PD	PD	PD	2	c				
7745	Maltodextrin	Maltodextrine	st	/	/	-	24.85	32.63	+	24.89	32.74	+	25.21	32.26	+	25.15	32.16	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PD	PD	PD	PD	2	c				
7746	Non-fat dry milk powder	Poudre de lait écrémé	+p	-	+	+	-	/26.74*	-	/32.07*	i/+*	-	/27.47*	-	/31.83*	i/+*	32.92	-	+	28.74	32.94	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	2	c
7747	Non-fat dry milk powder	Poudre de lait écrémé	+p	-	+	+	-26.47*	-	/32.11*	i/+*	27.74	36.23	+	/27.10*	-	/32.23*	i/+*	27.05	31.92	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	2	c		
7748	Whey	Lactosérum	st	/	/	-	-	32.82	-	-	32.51	-	-	32.53	-	-	32.12	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
7749	Whey	Lactosérum	st	/	/	-	-	33.01	-	-	32.95	-	-	32.50	-	-	32.46	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
7750	Whey	Lactosérum	+p	-	+	+	29.82	32.56	+	25.59	32.41	+	28.09	31.72	+	25.12	35.92	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	2	c				
7751	Maltodextrin	Maltodextrine	+p	-	+	+	21.04	33.95	+	22.27	32.62	+	20.9	32.47	+	22.40	31.88	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	2	c				
7752	Whey	Lactosérum	st	/	/	-	30.02	-	+	28.04	35.31	+	30.88	-	+	29.21	32.18	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PD	PD	PD	PD	2	c				
7753	Non-fat dry milk powder	Poudre de lait écrémé	+p	-	+	+	-	/24.59*	-	/32.52*	i/+*	25.92	39.70	+	28.37	-	+	29.28	-	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PA	PA	PA	PA	2	c		
7754	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	27.71	39.89	+	38.46	-	+	29.28	35.64	+	27.38	36.38	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PD	PD	PD	PD	2	c				
7755	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	27.24	34.89	+	26.41	36.15	+	27.25	31.84	+	26.61	32.44	+	+p	-	Cronobacter spp.	-	Cronobacter spp.	/	+	+	+	+	+	PD	PD	PD	PD	2	c				
8021	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	-	33.12	-	-	32.47	-	-	33.58	-	-	32.51	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8022	Maltodextrin	Maltodextrine	st	/	/	-	-	32.85	-	-	33.08	-	-	32.97	-	-	32.81	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8023	Whey	Lactosérum	st	/	/	-	-	32.39	-	-	32.45	-	-	32.29	-	-	32.15	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8024	Whey	Lactosérum	st	/	/	-	-	32.44	-	-	32.05	-	-	32.13	-	-	32.11	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	2	c					
8025	Whey	Lactosérum	st	/	/	-	-	31.78	-	-	32.14	-	-	32.27	-	-	33.00	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8114	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	-	34.10	-	-	34.23	-	-	34.25	-	-	33.95	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8115	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	-	32.86	-	-	34.27	-	-	33.96	-	-	34.58	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8116	Milk powder ingredient	Ingredient poudre de lait	st	/	/	-	-	34.11	-	-	33.80	-	-	34.05	-	-	33.75	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8117	Milk powder ingredient	Ingredient poudre de lait	st	/	/	-	-	34.00	-	-	33.08	-	-	33.82	-	-	33.4	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					
8118	Maltodextrin	Maltodextrine	st	/	/	-	-	33.86	-	-	34.03	-	-	33.63	-	-	33.31	-	st	/	/	/	/	st	-	-	-	-	NA	NA	NA	NA	2	c					

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																													
Sample N°	Product	Product (French name)	ISO 22964*	Alternative method: iQ-Check <i>Cronobacter</i> spp																						Category	Type		
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C + enrichment broth - Storage for 72h at 5°C ± 3°C																									
				PCR												RAPID [®] Sakazakii (direct streaking 10 * µl)		All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol						
				Easy protocol												Confirmation Without purification step			APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast			APF Fast with FDRS	
				APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Oxidase												Bio-chemical gallery (API ID32)
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result															
7720	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	-	27.2	34.5	+	24.83	32.22	+	27.36	32.58	+	25.57	33.11	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	a
7721	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	+	26.03	34.81	+	27.8	32.49	+	25.59	33.24	+	28.8	33.71	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7722	Infant cereals without probiotic 5 cereals	Céréales infantiles sans probiotique 5 céréales	+	21.55	39.28	+	22.38	34.07	+	21.54	-	+	23.46	34.25	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7723	Infant cereals without probiotic vanilla (10 months+)	Céréales infantiles sans probiotique babivanille (10+ mois)	+	26.24	34.41	+	36.65	35.41	+	25.97	34.03	+	27.47	33.63	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7724	Infant cereals without probiotic cocoa biscuit	Céréales infantiles sans probiotique chocobiscuité	+	22.83	-	+	23.75	33.39	+	22.75	-	+	24.59	34.71	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7725	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	+	26.55	33.41	+	26.74	33.97	+	26.29	33.05	+	27.75	33.17	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7726	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	+	21.28	-	+	23.42	36.88	+	20.52	-	+	24.89	33.36	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7727	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	+	24.78	35.58	+	26.9	32.3	+	25.23	34.84	+	27.52	33.33	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7728	Infant formula stage 2 organic	Poudre de lait infantile bio 2ème âge sans probiotique	+	21.21	33.46	+	23.64	38.5	+	20.77	40.59	+	25.14	35.92	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7729	Follow-up infant formula	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	+	21.01	-	+	24.74	34.75	+	20.72	40.73	+	26.77	33.53	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7730	Infant formula stage 1	Poudre de lait infantile sans probiotique premier âge	+	22.69	34.71	+	23.53	-	+	22.32	41.63	+	24.21	3.23	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
7731	Infant formula stage 2	Poudre de lait infantile sans probiotique 2ème âge	+	24.86	36.77	+	27.64	31.83	+	23.44	33.2	+	27.79	32.13	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	a
8019	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	-	25.41	35.77	+	25.65	35.74	+	24.4	34.04	+	24.84	34.24	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	a
7732	Infant formula with probiotics stage 2 (B.infantis 8,3.10 ⁴ CFU/g)	Poudre de lait avec probiotiques 2ème âge (B.infantis 8,3.10 ⁴ CFU/g)	+	22.96	38.96	+	24.59	32.27	+	22.88	-	+	25.35	33.27	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7733	Infant formula with probiotics thick formula with starch stage 2 (Bifidobacteria 8,3.10 ⁴ CFU/g)	Poudre de lait avec probiotiques formule épaissie amidon 2ème âge (Bifidobacteries 8,3.10 ⁴ CFU/g)	-	23.16	39.05	+	23.17	34.91	+	23.75	42.92	+	25.37	34.6	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	b
7734	Infant formula with probiotics thick formula stage 2 (B.infantis 3,7.10 ⁶ CFU/g)	Poudre de lait avec probiotiques actigest formule epaisse 2ème âge (B.infantis 8,3.10 ⁴ CFU/g)	+	22.33	38.65	+	23.82	31.76	+	22.74	38.08	+	24.33	33.39	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b

* Analyses performed according to the COFRAC accreditation (Accreditation Testing n°1-0144, scope available on www.cofrac.fr)

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																													
Sample N°	Product	Product (French name)	ISO 22964*	Alternative method: iQ-Check <i>Cronobacter</i> spp																									
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C + enrichment broth - Storage for 72h at 5°C ± 3°C																									
				PCR												RAPID [®] <i>Sakazakii</i> (direct streaking 10 * µl)		All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol						
				Easy protocol												Typical colonies	Confirmation Without purification step												
				APF			APF with FDRS			APF Fast			APF Fast with FDRS				Oxidase		Bio-chemical gallery (API ID32)	APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS		
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result															
7735	Infant formula with probiotics stage 2 (L. reuteri 9,8.10 ⁵ CFU/g)	Poudre de lait avec probiotiques relais 2ème âge (L. reuteri 9,8.10 ⁵ CFU/g)	-	22.85	-	+	25.62	31.57	+	24.32	-	+	26.77	35.88	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	b
7736	Infant formula with probiotics thick formula stage 2 (Bifidobacteria 1,5.10 ³ CFU/g)	Poudre de lait avec probiotiques formule épaisse 2ème âge (Bifidobactéries 1,5.10 ³ CFU/g)	+	22.83	-	+	27.29	33.28	+	23.12	-	+	27.59	34.07	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7738	Infant cereals with probiotics vanilla (B. lactis 5,3.10 ⁵ CFU/g)	Céréales infantiles vanille avec probiotiques (B.lactis 5,3.10 ⁵ CFU/g)	+	35.03	33.84	+	34.64	33.53	+	31.75	33.02	+	32.82	32.27	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7739	Infant cereals with probiotics 5 cereals (B. lactis 2,7.10 ⁶ CFU/g)	Céréales infantiles 5 céréales avec probiotiques (B.lactis 2,7.10 ⁶ CFU/g)	+	28.36	33.74	+	28.56	34.48	+	27.86	35.96	+	26.03	32.42	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7740	Infant cereals with probiotics cocoa vanilla (B. lactis 4,5.10 ⁶ CFU/g)	Céréales infantiles vanille chocolat au lait avec probiotiques (B.lactis 4,5.10 ⁶ CFU/g)	+	25.68	35.7	+	23.54	33.96	+	23.74	-	+	22.33	24.14	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7741	Infant cereals with probiotics biscuit (B. lactis 1,1.10 ⁶ CFU/g)	Céréales infantiles saveur biscuit avec probiotiques (B.lactis 1,1.10 ⁶ CFU/g)	+	28.27	32.35	+	31.36	32.91	+	26.74	32.58	+	27.86	32.11	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7742	Infant cereals with probiotics oat and wheat (B. lactis 2,7.10 ⁶ CFU/g)	Céréales infantiles avoine et blé avec probiotiques (B.lactis 2,7.10 ⁶ CFU/g)	+	28.8	33.92	+	28.48	35.02	+	27.46	37.3	+	25.65	42.2	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7743	Infant cereals with probiotics biscuit and nut (B. lactis 4,0.10 ⁶ CFU/g)	Céréales infantiles noisette biscuit avec probiotiques (B.lactis 4,0.10 ⁶ CFU/g)	+	25.02	32.81	+	28.08	32.43	+	26.26	33.03	+	26.72	32.01	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	b
7744	Maltodextrin	Maltodextrine	-	22.2	31.82	+	24.07	31.97	+	22.27	32.35	+	24.05	32.08	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	c
7745	Maltodextrin	Maltodextrine	-	26.09	32.8	+	27.11	33.52	+	24.12	31.66	+	24.95	31.74	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	c
7746	Non-fat dry milk powder	Poudre de lait écrémé	+	28.04	-	+	33.78	-	+	26.68	-	+	26.16	-	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	c
7747	Non-fat dry milk powder	Poudre de lait écrémé	+	21.85	-	+	-/21.49*	-/34.14*	i/+*	25.73	-	+	24.7	-	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	c
7750	Whey	Lactosérum	+	27.15	31.37	+	27.76	31.26	+	22.23	-	+	26.67	34.76	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	c
7751	Maltodextrin	Maltodextrine	+	23.29	32.05	+	24.82	31.42	+	22.48	41.27	+	22.46	31.59	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	c
7752	Whey	Lactosérum	-	25.62	31.72	+	30.79	32.44	+	25.04	31.46	+	28.91	32.73	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	c

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS AND INGREDIENTS (up to 375 g sample size) (Study realised by ADRIA Développement)																													
Sample N°	Product	Product (French name)	ISO 22964*	Alternative method: iQ-Check <i>Cronobacter</i> spp																									
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C + enrichment broth - Storage for 72h at 5°C ± 3°C																									
				PCR												RAPID [®] <i>Sakazakii</i> (direct streaking 10 * µl)		All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol						
				Easy protocol												Confirmation Without purification step			APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS			
APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Oxidase	Bio-chemical gallery (API ID32)															
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result				Target Cq	I.C. Cq	Final result												
7753	Non-fat dry milk powder	Poudre de lait écrémé	+	21.95	-	+	30.55	-	+	25.62	-	+	26.58	-	+	+p	-	<i>Cronobacter</i> spp.	+	+	+	+	+	PA	PA	PA	PA	2	c
7754	Non-fat dry milk powder	Poudre de lait écrémé	-	24.7	-	+	33.18	-	+	25.13	-	+	27.47	-	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	c
7755	Non-fat dry milk powder	Poudre de lait écrémé	-	23.55	33.1	+	30.01	33.16	+	25.01	-	+	27.15	40.56	+	+p	-	NI/ <i>Cronobacter</i> spp.	+	+	+	+	+	PD	PD	PD	PD	2	c

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ-Check <i>Cronobacter</i> spp																								Category	Type					
				30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h ± 2h at 37°C																														
		CCI	Oxidase	Identification	Result	PCR												RAPID <i>Sakazakii</i> (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol				
						Easy protocol												Without purification step		With purification step														
						APF		APF with FDRS		APF Fast		APF Fast with FDRS		Typical colonies	Oxidase	Biochemical gallery (API ID32)	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast			APF Fast with FDRS	APF	APF with FDRS	APF Fast			APF Fast with FDRS				
Target Cq	I.C. Cq					Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq															I.C. Cq	Final result					
1d	Process Water (Preclean - Port 1) - Spice Manufacturer	st	/	/	-	-	33.17	-	-	32.74	-	-	33.36	-	-	32.54	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
2d	Process Water (Post clean - Port 1) - Spice Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	21.72	31.97	+	19.6	32.07	+	22.09	32.07	+	20.7	32.17	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a
3d	Process Water (Preclean - Port 2)-Milk and Dairy Manufacturer	st	/	/	-	-	33.06	-	-	32.55	-	-	32.83	-	-	32.53	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
4d	Process Water (Post clean - Port 2)-Milk and Dairy Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	20.85	32.34	+	19.76	32.28	+	21.58	32.22	+	20.84	32.12	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a
5d	Process Water (Preclean - Port 3)-Milk and Dairy Manufacturer	st	/	/	-	-	32.87	-	-	32.54	-	-	32.81	-	-	32.65	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
6d	Process Water (Post clean - Port 3)-Milk and Dairy Manufacturer	st	/	/	-	-	33.13	-	-	33.21	-	-	33.21	-	-	32.55	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
7d	Process Water (Preclean - Port 4)-Spice Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	20.28	32.3	+	19.86	32.1	+	21.64	32.57	+	20.73	32.19	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a
8d	Process Water (Post clean - Port 4)-Spice Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	20.52	32.46	+	20.09	32.17	+	21.62	32.5	+	20.9	32.19	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a
9d	Process Water (Preclean - Port 5)-Milk and Dairy Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	22.11	32.3	+	20.31	32.14	+	22.02	32.08	+	21.32	32.05	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a
10d	Process Water (Post clean - Port 5)-Milk and Dairy Manufacturer	st	/	/	-	-	33.06	-	-	32.89	-	-	32.96	-	-	32.81	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
11d	Process Water (Preclean - Hose 1)-Spice Manufacturer	st	/	/	-	-	33.13	-	-	33.11	-	-	33.07	-	-	32.53	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
12d	Process Water (Post clean - Hose 1)-Spice Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	20.8	32.2	+	19.59	32.16	+	21.74	32.3	+	20.98	32.02	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a
13d	Process Water (Preclean - Hose 2)-Spice Manufacturer	st	/	/	-	-	32.78	-	-	32.86	-	-	32.84	-	-	32.89	-	st	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	a	
14d	Process Water (Post clean - Hose 2)-Spice Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	20.14	32.2	+	19.69	32.27	+	21.65	32.21	+	21.01	32.32	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	a

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)

Sample N°	Product	Alternative method: iQ-Check <i>Cronobacter</i> spp																												Category	Type									
		ISO 22964				30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h ± 2h at 37°C																																		
		CCI	Oxidase	Identification	Result	PCR												RAPID <i>Sakazakii</i> (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol				Agreement Easy Protocol												
						Easy protocol						Confirmation						Without purification step		With purification step																				
						APF		APF with FDRS		APF Fast		APF Fast with FDRS		Typical colonies	Oxidase	Biochemical gallery (API ID32)	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast			APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS												
Target Cq	I.C. Cq					Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq																I.C. Cq	Final result										
15d	Process Water (Preclean - Hose 3)-Milk and Dairy Manufacturer	+M	-	<i>Cronobacter</i> spp.	+	20.37	32.33	+	19.84	32.28	+	21.83	32.69	+	21.07	32.03	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	+	+	PA	PA	PA	PA	3	a				
16d	Process Water (Post clean - Hose3)-Milk and Dairy Manufacturer	st	/	/	-	-	32.84	-	-	32.78	-	-	33.06	-	-	32.94	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	3	a				
17d	Process Water (Preclean - Hose 4)-Spice Manufacturer	st	/	/	-	-	37.17	-	-	32.78	-	-	33.12	-	-	32.93	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	3	a			
18d	Process Water (Post clean - Hose 4)-Spice Manufacturer	st	/	/	-	-	36.63	-	-	33.12	-	-	33.09	-	-	32.98	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	a		
19d	Process Water (Preclean - Hose 5)-Milk and Dairy Manufacturer	st	/	/	-	-	36.93	-	-	32.84	-	-	33.14	-	-	32.89	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	a		
20d	Process Water (Post clean - Hose 5)-Milk and Dairy Manufacturer	st	/	/	-	-	36.13	-	-	33.04	-	-	33.19	-	-	32.74	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	a	
21d	Process Water (Preclean - Port 6)-Milk and Dairy Manufacturer	st	/	/	-	-	34.88	-	-	32.6	-	-	33	-	-	33.12	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	a	
22d	Process Water (Post clean - Port 6)-Milk and Dairy Manufacturer	st	/	/	-	-	33.17	-	-	32.65	-	-	33.01	-	-	32.83	-	st	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	a
1e	Sponge - Conveyer belt (Preclean)-Milk and Dairy Manufacturer	-	/	/	-	-	31.7	-	-	31.38	-	-	32.04	-	-	31.97	-	-	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	b	
4e	Swab - Drain #1 (Post-clean)-Spice Manufacturer	-	/	/	-	-	31.73	-	-	31.61	-	-	31.97	-	-	31.85	-	-	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	b
7e	Swab - Drain #2 (Post-clean)-Spice Manufacturer	-	/	/	-	-	31.81	-	-	31.58	-	-	31.87	-	-	31.94	-	-	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	b
8e	Sponge - Employee glove - post shift (Left)-Milk and Dairy Manufacturer	-	/	/	-	-	32.08	-	-	31.58	-	-	32.03	-	-	31.85	-	-	/	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	b
9e	Sponge - Employee shoe - post shift (Right)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	18.06	39.02	+	18.15	35.02	+	17.4	3926	+	17.78	36.32	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	b	

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ-Check <i>Cronobacter</i> spp																								Category	Type							
				30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h ± 2h at 37°C																																
		CCI	Oxidase	Identification	Result	PCR												RAPID <i>Sakazakii</i> (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol						
						Easy protocol						Confirmation						Typical colonies	Without purification step		With purification step			APF	APF with FDRS	APF Fast	APF Fast with FDRS									
						APF		APF with FDRS		APF Fast		APF Fast with FDRS		Without purification step		With purification step																				
Target Cq	I.C. Cq					Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS			APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS							
12e	Sponge - Doorhandle #4 (preclean)-Spice Manufacturer	-	/	/	-	-	31.9	-	-	31.64	-	-	31.6	-	-	31.77	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	3	b
13e	Sponge - Doorhandle #4 (Post-clean)-Spice Manufacturer	-	/	/	-	-	32.11	-	-	31.82	-	-	32.42	-	-	32.07	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	3	b
14e	Sponge - Trash Can #6-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	18.25	39.76	+	18.24	35.64	+	17.8	39.64	+	17.97	36.06	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
15e	Swab - Mixing Blade #1 (Post-clean)-Spice Manufacturer	-	/	/	-	-	31.95	-	-	31.47	-	-	32.17	-	-	31.87	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	3	b
16e	Swab - Mixing Blade #2 (Preclean)-Spice Manufacturer	-	/	/	-	-	31.83	-	-	31.6	-	-	31.81	-	-	32.04	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	3	b
19e	Swab - Doorhandle #2 (preclean)-Spice Manufacturer	-	/	/	-	-	31.34	-	-	31.64	-	-	31.74	-	-	31.93	-	-	/	/	/	/	-	-	-	-	-	-	-	-	NA	NA	NA	NA	3	b
21e	Swab - Doorhandle #6 - Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	22.04	36.12	+	19.14	32.27	+	19.76	31.91	+	19.71	32.17	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
22e	Swab - Employee glove - post shift (Left) - Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	21.62	35.89	+	19.27	32.9	+	19.63	91.86	+	19.51	32.08	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
23e	Sponge - Mixing Blade #8 (Preclean) - Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	21.35	36.47	+	19.48	33.12	+	19.44	31.91	+	19.39	32.12	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
24e	Swab - Employee glove - post shift (Right) - Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	21.05	35.42	+	20.12	34.18	+	19.6	31.72	+	19.43	31.85	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
25e	Sponge - Employee shoe - post shift (Left) - Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	20.93	35.37	+	20.68	34.98	+	19.55	31.59	+	19.33	31.77	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
26e	Sponge - Employee shoe - post shift (Right) - Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	20.72	35.16	+	21.06	34.99	+	19.45	31.54	+	19.3	31.74	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
27e	Swab - Drain #4 (Preclean) - Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	20.45	34.09	+	21.2	35.59	+	19.9	31.37	+	19.51	32.03	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		
28e	Sponge - Trash Can #4 - Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	19.9	33.71	+	21.61	35.57	+	19.5	31.81	+	19.7	31.77	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b		

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ-Check <i>Cronobacter</i> spp																								Category	Type					
				30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h ± 2h at 37°C																														
		CCI	Oxidase	Identification	Result	PCR												RAPID'Sakazakii (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol				
						Easy protocol						Confirmation						Typical colonies	Without purification step		With purification step			APF	APF with FDRS	APF Fast	APF Fast with FDRS			APF	APF with FDRS	APF Fast	APF Fast with FDRS	
						APF		APF with FDRS		APF Fast		APF Fast with FDRS		Confirmation		Oxidase	Biochemical gallery (API ID32)		Oxidase	Biochemical gallery (API ID32)														
Target Cq	I.C. Cq					Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result																			
29e	Sponge - Conveyor belt #3 (Preclean) - Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	19.42	32.68	+	19.63	33.47	+	19.81	31.86	+	19.35	32.1	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	b
1f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	26.77	33.89	+	25.17	30.4	+	27.01	35.62	+	27.06	35.64	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
2f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.34	32.33	+	23.44	30.62	+	24.25	33.4	+	24.58	34.39	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
3f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	26.44	33.07	+	25.31	32.23	+	26.72	35.19	+	27.1	35.71	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
4f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	-	/	/	-	-	32.65	-	-	32.44	-	-	33.05	-	-	32.38	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
5f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.37	32.37	+	23.55	30.96	+	24.16	33.13	+	24.61	33.59	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
6f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	-	/	/	-	-	32.47	-	-	32.32	-	-	32.56	-	-	32.75	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
7f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	-	/	/	-	-	32.27	-	-	32.85	-	-	32.25	-	-	32.86	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
8f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.36	32.8	+	23.99	31.19	+	24.2	33.57	+	24.57	33.36	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
9f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	-	/	/	-	-	32.27	-	-	32.78	-	-	32.57	-	-	32.42	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
10f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	30.83	32.68	+	29.94	31.62	+	30.73	34.46	+	31.48	34.25	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
11f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	26.66	33.37	+	26.4	33.95	+	26.69	35.21	+	26.82	35.8	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
12f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.41	32.99	+	24.06	31.98	+	24.24	32.62	+	24.45	32.64	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
13f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	-	/	/	-	-	32.49	-	-	32.38	-	-	32.64	-	-	32.74	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
14f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	-	/	/	-	-	32.41	-	-	32.65	-	-	32.41	-	-	32.63	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964		Alternative method: iQ-Check <i>Cronobacter</i> spp																								Category	Type					
				30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h ± 2h at 37°C																														
		CCI	Oxidase	Identification	Result	PCR												RAPID <i>Sakazakii</i> (direct streaking 10 µl)				Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final Result Easy protocol						Agreement Easy Protocol				
						Easy protocol						Confirmation						Without purification step		With purification step														
						APF			APF with FDRS			APF Fast			APF Fast with FDRS			Typical colonies	Oxidase	Biochemical gallery (API ID32)	Oxidase			Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast			APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS
Target Cq	I.C. Cq					Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result																			
15f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	-	/	/	-	-	32.44	-	-	32.66	-	-	32.24	-	-	32.86	-	-	/	/	/	/	-	-	-	-	-	-	NA	NA	NA	NA	3	c
16f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	31.32	33.13	+	30.76	32.66	+	31.3	33.62	+	31.67	34.23	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
17f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.41	32.92	+	24.26	31.6	+	24.3	32.72	+	24.54	33.46	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
18f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	26.58	32.86	+	26.15	32.3	+	26.77	35.41	+	26.92	35.58	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
19f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	-	/	/	-	-	32.6	-	-	32.29	-	-	32.3	-	-	32.36	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
20f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	26.54	33.29	+	26.05	31.94	+	26.47	-	+	26.69	35.43	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
21f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.33	32.71	+	24.18	31.29	+	24.2	33.33	+	24.46	33.54	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
22f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.28	32.66	+	24.26	31.78	+	24.19	32.86	+	24.45	33.18	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
23f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+1/2	-	<i>Cronobacter</i> spp.	+	24.42	31.67	+	24.27	32.49	+	24.22	33.38	+	24.37	34.46	+	+M	-	<i>Cronobacter</i> spp.	-	<i>Cronobacter</i> spp.	/	+	+	+	+	+	PA	PA	PA	PA	3	c
24f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	-	/	/	-	-	32.42	-	-	32.53	-	-	32.19	-	-	32.5	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	
25f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	-	/	/	-	-	32.39	-	-	32.79	-	-	32.35	-	-	32.35	-	-	/	/	/	/	-	-	-	-	-	NA	NA	NA	NA	3	c	

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)																														
Sample N°	Product	ISO 22964	Alternative method: iQ-Check <i>Cronobacter</i> spp																											
			30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h +/- 2h at 37°C + enrichment broth - Storage for 48h at 5°C ± 3°C																											
			PCR												RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final Result Easy protocol -				Agreement Easy Protocol -				Category	Type
			Easy protocol												Confirmation						Typical colonies									
			APF			APF with FDRS			APF Fast			APF Fast with FDRS			Without purification step															
Target Cq	I.C. Cq	Final result	Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)	APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS			APF Fast	APF Fast with FDRS							
2d	Process Water (Post clean - Port 1) - Spice Manufacturer	+	21.16	32.4	+	20.55	31.85	+	20.62	31.75	+	20.23	31.63	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
4d	Process Water (Post clean - Port 2)-Milk and Dairy Manufacturer	+	21.07	32.26	+	20.61	32.25	+	20.4	31.87	+	20.24	31.57	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
7d	Process Water (Preclean - Port 4)-Spice Manufacturer	+	20.62	32.24	+	20.44	32.31	+	20.35	31.55	+	20.13	31.56	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
8d	Process Water (Post clean - Port 4)-Spice Manufacturer	+	20.81	32.52	+	20.35	31.81	+	20.44	31.86	+	19.8	31.53	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
9d	Process Water (Preclean - Port 5)-Milk and Dairy Manufacturer	+	20.84	32.5	+	20.3	32.09	+	20.31	32.05	+	19.78	31.55	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
12d	Process Water (Post clean - Hose 1)-Spice Manufacturer	+	20.8	32.14	+	20.41	32.13	+	20.41	31.7	+	19.74	31.27	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
14d	Process Water (Post clean - Hose 2)-Spice Manufacturer	+	20.59	32.41	+	20.3	32.16	+	20.47	31.69	+	19.63	31.64	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
15d	Process Water (Preclean - Hose 3)-Milk and Dairy Manufacturer	+	20.83	32.19	+	20.38	31.85	+	20.51	31.59	+	19.94	31.45	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	a			
9e	Sponge - Employee shoe - post shift (Right)-Milk and Dairy Manufacturer	+	18.37	41.84	+	19.98	34.74	+	17.82	39.72	+	19.63	34.63	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
14e	Sponge - Trash Can #6-Milk and Dairy Manufacturer	+	18.23	41.07	+	18.55	38.54	+	17.91	39.89	+	18.2	36.72	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
21e	Swab - Doorhandle #6 - Milk and Dairy Manufacturer	+	19.33	34.39	+	18.2	32.08	+	18.38	31.76	+	18.78	32.13	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
22e	Swab - Employee glove - post shift (Left) - Spice Manufacturer	+	19.4	33.87	+	18.57	32.4	+	18.74	31.78	+	19.12	32.03	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
23e	Sponge - Mixing Blade #8 (Preclean) - Milk and Dairy Manufacturer	+	19.97	33.46	+	18.77	32.79	+	19.27	31.7	+	18.92	31.86	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
24e	Swab - Employee glove - post shift (Right) - Spice Manufacturer	+	19.71	33.18	+	18.89	33.16	+	19.08	31.55	+	18.61	32.33	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
25e	Sponge - Employee shoe - post shift (Left) - Spice Manufacturer	+	19.74	33.74	+	19.44	33.26	+	18.91	31.95	+	18.65	31.68	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
26e	Sponge - Employee shoe - post shift (Right) - Spice Manufacturer	+	19.34	34.12	+	19.73	33.36	+	18.42	31.73	+	18.71	32.07	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
27e	Swab - Drain #4 (Preclean) - Milk and Dairy Manufacturer	+	19.92	33.55	+	19.76	33.76	+	19	31.73	+	18.82	31.61	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
28e	Sponge - Trash Can #4 - Milk and Dairy Manufacturer	+	20.17	33.55	+	19.99	33.95	+	19.11	31.81	+	18.82	31.79	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
29e	Sponge - Conveyer belt #3 (Preclean) - Milk and Dairy Manufacturer	+	19.99	33.45	+	20.41	33.85	+	18.9	32	+	19.52	31.59	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	b			
1f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	25.5	34.59	+	23.52	30.11	+	25.33	32.73	+	26.81	-	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c			
2f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+	27.43	35.7	+	25.27	31.99	+	27.98	-	+	26.42	32.62	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c			
3f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	25.21	33.21	+	23.68	31.1	+	25.39	33.84	+	24.83	30.86	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c			
5f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	32.29	33.49	+	25.69	32.15	+	33.05	-	+	26.04	34.24	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c			

ENVIRONMENTAL SAMPLES (Study realized by Q-Laboratories Inc.)

Sample N°	Product	ISO 22964	Alternative method: iQ-Check <i>Cronobacter</i> spp																					Category	Type				
			30g + 270 mL BPW or Sampling device + 10 mL (Swab), + 100 mL (sponge) + 225 mL (wipe) - 18h +/- 2h at 37°C + enrichment broth - Storage for 48h at 5°C ± 3°C																										
			PCR												RAPID'Sakazakii (direct streaking 10 µl)				All confirmatory tests	Final Result Easy protocol -						Agreement Easy Protocol -			
			Easy protocol												Typical colonies	Confirmation		APF		APF with FDRS	APF Fast	APF Fast with FDRS	APF			APF with FDRS	APF Fast	APF Fast with FDRS	
			APF			APF with FDRS			APF Fast			APF Fast with FDRS				Without purification step													
Target Cq	I.C. Cq	Final result	Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)																
8f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+	32.12	33.92	+	26.3	32.48	+	32.37	35.41	+	25.86	-	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
10f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	27.61	34.33	+	30.55	32.27	+	27.31	-	+	29.57	32.31	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
11f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	25.24	32.99	+	24.62	32.31	+	25.09	32.69	+	23.66	31.78	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
12f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+	32.72	34.24	+	31.39	32.5	+	31.79	33.72	+	29.58	30.77	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
16f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+	25.44	34.72	+	27.16	33.27	+	25.1	32.31	+	26.05	34.53	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
17f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	32.62	33.32	+	25.04	33.01	+	32.36	34.34	+	23.9	32.6	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
18f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+	25.12	32.71	+	24.96	32.57	+	24.89	33.71	+	25.25	34.54	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
20f	Dust (floor sweepings & dryer lint)-Milk and Dairy Manufacturer	+	32.44	33.874	+	31.89	32.88	+	32.03	33.71	+	32.53	34.68	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
21f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	25.17	34.01	+	25.05	32.99	+	24.93	33.25	+	25.28	33.1	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
22f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	27	32.2	+	25.13	33.29	+	27.21	34.37	+	25.18	32.65	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		
23f	Dust (floor sweepings & dryer lint)-Spice Manufacturer	+	27.37	34.56	+	25.12	32.66	+	27.29	34.04	+	25.22	32.27	+	+M	-	<i>Cronobacter</i> spp.	+	+	+	+	PA	PA	PA	PA	3	c		

Matrix : Infant formula with probiotics – 375g

Strain : *Cronobacter sakazakii* Ad1418

Seeding protocol with lyophilized strain storage for 2 weeks at ambient temperature

Unpaired

Total viable count: 2,8.10⁸ CFU/g

Lactic count : 1,7.10⁶ CFU/g

Sample N°	Level	Inoculation level (cfu/10g)	ISO 22964*				iQ-Check <i>Cronobacter</i> spp. method 375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C±1°C																							
			CCI	Oxidase	Identification	Result	Positive Results/Total (ISO22964)	PCR												RAPID [®] <i>Sakazakii</i> (direct streaking 10 µl)	All confirmatory tests	iQ-Check <i>Cronobacter</i> spp								
								Easy protocol														Typical colonies	Final Result Easy protocol				Positive Results/Total Easy protocol			
								APF			APF with FDRS			APF Fast			APF Fast with FDRS						APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS								
8205	0	/	st	/	/	-	0 / 5	-	33.51	-	-	34.75	-	-	33.02	-	-	32.97	-	st	-	-	-	-	0 / 5	0 / 5	0 / 5	0 / 5		
8206			st	/	/	-		-	33.97	-	-	33.84	-	-	33	-	-	32.5	-	st	-	-	-	-						
8207			st	/	/	-		-	33.47	-	-	33.51	-	-	32.58	-	-	32.53	-	st	-	-	-	-						
8208			st	/	/	-		-	33.71	-	-	33.28	-	-	33.11	-	-	33.25	-	st	-	-	-	-						
8209			st	/	/	-		-	33.81	-	-	33.2	-	-	33.05	-	-	32.63	-	st	-	-	-	-						
8268	Low	1.9	st	/	/	-	14/20	24.12	34.48	+	23.11	37.31	+	22.37	38.00	+	23.34	-	+	+p	+	+	+	+	15/20	15/20	15/20	15/20		
8269			+p	-	+	+		26.35	33.84	+	24.91	35.02	+	25.63	33.08	+	24.05	-	+	+p	+	+	+	+						
8270			st	/	/	-		28.25	32.55	+	26394	32.93	+	26.90	33.87	+	26.12	35.05	+	+p	+	+	+	+						
8271			+p	-	+	+		29.00	32.47	+	30.32	32.64	+	28.63	33.07	+	29.38	33.09	+	+p	+	+	+	+						
8272			+p	-	+	+		-	33.13	+	-	33.13	-	-	33.26	-	-	33.79	-	st	-	-	-	-						
8273			+p	-	+	+		27.54	33.75	+	27.59	34.51	+	26.31	33.43	-	26.54	33.14	+	+p	+	+	+	+						
8274			st	/	/	-		27.03	32.03	+	29.13	32.97	+	27.04	32.62	+	28.97	33.07	+	+p	+	+	+	+						
8275			st	/	/	-		36.75	33.40	+	34.17	32.81	+	32.43	33.03	+	33.30	33.10	+	+p	+	+	+	+						
8276			+p	-	+	+		29.40	32.96	+	28.98	33.19	+	27.30	32.78	+	27.00	33.31	+	+p	+	+	+	+						
8277			st	/	/	-		31.57	32.26	+	32.41	32.22	+	31.29	33.50	+	30.95	32.40	+	+d/+	+	+	+	+						
8278			+p	-	+	+		-	32.88	-	-	32.46	-	-	33.52	-	-	33.49	-	st	-	-	-	-						
8279			st	/	/	-		-	33.17	-	-	33.40	-	-	33.80	-	-	33.68	-	st	-	-	-	-						
8280			+p	-	+	+		27.93	33.22	+	27.02	32.75	+	27.31	34.49	+	26.44	33.98	+	+p	+	+	+	+						
8281			+p	-	+	+		32.11	33.02	+	32.97	32.43	+	33.30	33.23	+	31.91	32.79	+	+p	+	+	+	+						
8282			+p	-	+	+		27.43	40.61	+	25.97	33.16	+	26.35	40.09	+	25.21	33.54	+	+p	+	+	+	+						
8283			+p	-	+	+		-	33.87	-	-	32.61	-	-	34.13	-	-	33.52	-	st	-	-	-	-						
8284			+p	-	+	+		27.26	33.16	+	26.59	33.28	+	26.78	34.89	+	26.24	34.93	+	+p	+	+	+	+						
8285			+p	-	+	+		-	32.37	-	-	32.65	-	-	33.57	-	-	33.06	-	st	-	-	-	-						
8286			+p	-	+	+		31.47	32.34	+	32.31	32.32	+	30.58	33.10	+	31.87	33.23	+	+p	+	+	+	+						
8287	+p	-	+	+	28.93	33.40	+	28.19	33.52	+	26.84	-	+	26.28	33.29	+	+p	+	+	+	+									
8288	High	5.1	+p	-	+	+	5 / 5	25.62	33.98	+	27.64	32.22	+	24.31	-	+	27.14	32.7	+	+p	+	+	+	+	5 / 5	5 / 5	5 / 5	5 / 5		
8289			+p	-	+	+		24.17	35.39	+	24.92	35.49	+	22.87	42.19	+	24.15	36.77	+	+p	+	+	+	+						
8290			+p	-	+	+		23.43	-	+	25.41	32.87	+	24.37	43.39	+	25.14	34.39	+	+p	+	+	+	+						
8291			+p	-	+	+		26.77	33.27	+	26	32.49	+	24.48	40.06	+	25.72	34.19	+	+p	+	+	+	+						
8292			+p	-	+	+		25.25	35.13	+	26.04	33.56	+	26.3	34.06	+	25.95	34.89	+	+p	+	+	+	+						

* Analyses performed according to the COFRAC accreditation (Accreditation Testing n°1-0144, scope available on www.cofrac.fr)

Matrix: Process water
 Strain: *Cronobacter sakazakii* CCUG 28868
 Seeding 48 h at 5 ± 3°C
 Unpaired
 Total viable count: 1,1.10² CFU/g

(Q-Lab)

Level	Inoculation level (cfu/25g)	Sample N° (reference method)	ISO 22964				Positive Results/Total (ISO22964)	Sample N° (alternative method)	iQ-Check <i>Cronobacter</i> spp. method - Category 3 - 30g + 270 mL BPW - 18h ± 2h at 37°C																						
			CCI	Oxidase	Identification	Result			PCR												RAPID'Sakazakii (direct streaking 10 µl)	All confirmatory tests	iQ-Check <i>Cronobacter</i> spp								
									Easy protocol														Typical colonies	Final Result Easy protocol				Positive Results/Total Easy protocol			
									APF			APF with FDRS			APF Fast			APF Fast with FDRS						APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS
Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq	Final result	APF	APF with FDRS	APF Fast	APF Fast with FDRS	APF	APF with FDRS	APF Fast	APF Fast with FDRS									
0	/	2	st	/	/	-	0/5	2	-	33.59	-	-	32.24	-	-	32.54	-	-	32.89	-	st	-	-	-	-	0/5	0/5	0/5	0/5		
		18	st	/	/	-		9	-	33.64	-	-	33.24	-	-	32.42	-	-	34.27	-	st	-	-	-	-						
		21	st	/	/	-		14	-	33.61	-	-	33.12	-	-	32.74	-	-	35.24	-	st	-	-	-	-						
		25	st	/	/	-		21	-	33.93	-	-	33.62	-	-	32.5	-	-	32.5	-	st	-	-	-	-						
		28	st	/	/	-		24	-	33.67	-	-	33.37	-	-	32.63	-	-	32.71	-	st	-	-	-	-						
Low	1.5	1	st	/	/	-	10/20	1	-	33.62	-	-	31.99	-	-	32.65	-	-	32.69	-	st	-	-	-	-	9/20	9/20	9/20	9/20		
		3	+M	-	<i>Cronobacter</i> spp.	+		3	20.65	36.02	+	19.8	32.67	+	20.16	33.82	+	21.13	33.41	+	+M	+	+	+	+						
		5	+M	-	<i>Cronobacter</i> spp.	+		4	20.45	35.38	+	19.99	32.93	+	20.42	34.03	+	20.86	33.45	+	+M	+	+	+	+						
		6	+M	-	<i>Cronobacter</i> spp.	+		5	20.78	36.74	+	20.06	3.3	+	20.18	33.43	+	21.07	33.49	+	+M	+	+	+	+						
		7	st	/	/	-		6	20.78	35.88	+	20.1	33.78	+	20.28	33.7	+	20.5	34.12	+	+M	+	+	+	+						
		8	st	/	/	-		7	-	35.22	-	-	33.17	-	-	32.54	-	-	32.73	-	st	-	-	-	-						
		9	+M	-	<i>Cronobacter</i> spp.	+		8	-	33.51	-	-	33.23	-	-	32.56	-	-	32.53	-	st	-	-	-	-						
		11	st	/	/	-		10	20.36	38.88	+	19.71	36.15	+	19.71	34.86	+	26.12	40.44	+	+M	+	+	+	+						
		12	st	/	/	-		11	-	33.2	-	-	33.14	-	-	32.48	-	-	34.07	-	st	-	-	-	-						
		13	+M	-	<i>Cronobacter</i> spp.	+		12	-	33.83	-	-	33.3	-	-	32.84	-	-	34.96	-	st	-	-	-	-						
		15	+M	-	<i>Cronobacter</i> spp.	+		13	-	33.59	-	-	33.1	-	-	33.19	-	-	35.12	-	st	-	-	-	-						
		16	st	/	/	-		15	19.76	39.72	+	19.85	35.63	+	19.13	35.76	+	29.82	NA	+	+M	+	+	+	+						
		19	st	/	/	-		16	-	33.47	-	-	33.29	-	-	32.46	-	-	35.48	-	st	-	-	-	-						
		20	+M	-	<i>Cronobacter</i> spp.	+		17	21.02	37.98	+	20.13	36.28	+	20.81	34.23	+	20.5	34.37	+	+M	+	+	+	+						
		22	+M	-	<i>Cronobacter</i> spp.	+		18	20.46	37.3	+	19.39	35.08	+	20.09	34.87	+	19.41	34.52	+	+M	+	+	+	+						
		23	st	/	/	-		19	-	33.73	-	-	33.32	-	-	32.44	-	-	32.53	-	st	-	-	-	-						
26	st	/	/	-	20	-	33.8	-	-	33.49	-	-	32.77	-	-	32.71	-	st	-	-	-	-									
27	+M	-	<i>Cronobacter</i> spp.	+	23	-	33.82	-	-	33.36	-	-	32.69	-	-	32.81	-	st	-	-	-	-									
29	st	/	/	-	25	-	34.17	-	-	33.67	-	-	32.66	-	-	33.02	-	st	-	-	-	-									
30	+M	-	<i>Cronobacter</i> spp.	+	28	19.85	39.34	+	19.84	38.39	+	20.03	37.58	+	19.46	34.44	+	+M	+	+	+	+									
High	8.8	4	+M	-	<i>Cronobacter</i> spp.	+	5/5	22	20.16	37.49	+	19.63	36.14	+	19.84	34.9	+	19.46	34.59	+	+M	+	+	+	+	5/5	5/5	5/5	5/5		
		10	+M	-	<i>Cronobacter</i> spp.	+		26	20.29	38.81	+	19.46	36.5	+	20.78	33.96	+	19.1	36.34	+	+M	+	+	+	+						
		14	+M	-	<i>Cronobacter</i> spp.	+		27	19.92	38.54	+	19.49	38.59	+	20.09	34.72	+	19.13	35.68	+	+M	+	+	+	+						
		17	+M	-	<i>Cronobacter</i> spp.	+		29	19.87	41.34	+	19.8	38.2	+	19.75	37.98	+	19.11	35.08	+	+M	+	+	+	+						
		24	+M	-	<i>Cronobacter</i> spp.	+		30	18.14	NA	+	18.24	41.15	+	17.58	39.08	+	17.76	38.22	+	+M	+	+	+	+						

Appendix 6 – Inclusivity and exclusivity study: raw data

INCLUSIVITY (Initial validation, 2007)								
No	Genus	Species	N°	Origin	Inoculation Level CFU/ 225ml BPW + vancomycin	PCR		RAPID' Sakazakii
						Easy protocol	Standard protocol	
1	<i>Cronobacter</i>	<i>dublinensis</i>	DSM18705	Dairy Product	5	+19.86	+16.84	+
2	<i>Cronobacter</i>	<i>malonaticus</i>	DSM18702	Dairy Product	2	+19.08	+16.12	+
3	<i>Cronobacter</i>	<i>malonaticus</i>	Ad1708	Dairy Product	19	+33.32	+17.02	+
4	<i>Cronobacter</i>	<i>muytjensii</i>	CIP103581	/	11	+19.45	+16.21	+
5	<i>Cronobacter</i>	<i>sakazakii</i>	Ad939	Infant formula	2	+19.01	+16.01	+
6	<i>Cronobacter</i>	<i>sakazakii</i>	Ad940	Infant formula	4	+18.62	+16.21	+
7	<i>Cronobacter</i>	<i>sakazakii</i>	Ad941	Infant formula	7	+18.75	+15.67	+
8	<i>Cronobacter</i>	<i>sakazakii</i>	Ad942	Infant formula	8	+18.70	+16.1	+
9	<i>Cronobacter</i>	<i>sakazakii</i>	Ad943	Infant formula	1	+18.67	+15.51	+
10	<i>Cronobacter</i>	<i>sakazakii</i>	Ad944	Infant formula	1	+20.21	+15.93	+
11	<i>Cronobacter</i>	<i>sakazakii</i>	Ad945	Infant formula	0	+20.33	+16.01	+
12	<i>Cronobacter</i>	<i>sakazakii</i>	Ad946	Infant formula	1	+19.10	+15.84	+
13	<i>Cronobacter</i>	<i>sakazakii</i>	Ad947	Infant formula	4	+19.29	+15.87	+
14	<i>Cronobacter</i>	<i>sakazakii</i>	Ad948	Infant formula	10	+19.02	+15.62	+
15	<i>Cronobacter</i>	<i>sakazakii</i>	Ad949	Infant formula	7	+18.62	+16.12	+
16	<i>Cronobacter</i>	<i>sakazakii</i>	Ad950	Infant formula	11	+18.86	+16.25	+
17	<i>Cronobacter</i>	<i>sakazakii</i>	Ad951	Infant formula	7	+19.72	+16.79	+
18	<i>Cronobacter</i>	<i>sakazakii</i>	Ad952	Infant formula	9	+19.44	+16.25	+
19	<i>Cronobacter</i>	<i>sakazakii</i>	Ad953	Infant formula	3	+19.21	+16.02	+
20	<i>Cronobacter</i>	<i>sakazakii</i>	Ad963	Infant formula	3	+19.64	+15.89	+
21	<i>Cronobacter</i>	<i>sakazakii</i>	95	Infant formula	1	+19.71	+15.51	+
22	<i>Cronobacter</i>	<i>sakazakii</i>	Ad704	Infant formula	7	+19.86	+16.09	+
23	<i>Cronobacter</i>	<i>sakazakii</i>	Ad831	Infant formula	8	+19.04	+16.88	+
24	<i>Cronobacter</i>	<i>sakazakii</i>	Ad829	Infant formula	7	+19.37	+16.91	+
25	<i>Cronobacter</i>	<i>sakazakii</i>	Ad916	Infant formula	7	+19.31	+16.74	+
26	<i>Cronobacter</i>	<i>sakazakii</i>	Ad893	Infant formula	8	+19.45	+16.35	+
27	<i>Cronobacter</i>	<i>sakazakii</i>	Ad894	Infant formula	7	+20.35	+15.51	+
28	<i>Cronobacter</i>	<i>sakazakii</i>	Ad895	Infant formula	3	+19.19	+15.08	+
29	<i>Cronobacter</i>	<i>sakazakii</i>	Ad896	Infant formula	5	+19.82	+16.05	+
30	<i>Cronobacter</i>	<i>sakazakii</i>	Ad897	Infant formula	8	+19.46	+15.71	+
31	<i>Cronobacter</i>	<i>sakazakii</i>	Ad898	Infant formula	11	+19.51	+16.37	+
32	<i>Cronobacter</i>	<i>dublinensis lactaridi</i>	DSMZ18707 T	Dairy Product	2	+19.70	+15.70	+
33	<i>Cronobacter</i>	<i>dublinensis lausannensis</i>	DSMZ 18706 T	Dairy Product	1	+19.09	+16.10	+
34	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1418	Infant formula	9	+19.86	+15.77	+
35	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1419	Infant formula	5	+19.79	+15.33	+
36	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1420	Infant formula	13	+19.61	+15.15	+
37	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1421	Infant formula	11	+20.19	+15.37	+
38	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1424	Infant formula	3	+20.04	+15.75	+

INCLUSIVITY (Initial validation, 2007)								
No	Genus	Species	N°	Origin	Inoculation Level CFU/ 225ml BPW + vancomycin	PCR		RAPID' Sakazakii
						Easy protocol	Standard protocol	
39	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1425	Infant formula	1	+19.19	+15.63	+
40	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1426	Infant formula	38	+18.86	+14.92	+
41	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1427	Infant formula	1	+18.73	+16.50	+
42	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1428	Infant formula	10	+19.16	+16.30	+
43	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1429	Infant formula	1	+20.23	+15.83	+
44	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1430	Infant formula	8	+21.41	+15.84	+
45	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1431	Infant formula	17	+21.17	+15.96	+
46	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1432	Infant formula	10	+23.19	+16.04	+
47	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1433	Infant formula	8	+23.86	+16.91	+
48	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1434	Infant formula	5	+22.55	+16.22	+
49	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1435	Infant formula	14	+21.15	+16.67	+
50	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1436	Infant formula	4	+19.70	+16.64	+
51	<i>Cronobacter</i>	<i>turicensis</i>	Ad 1445	Infant formula	4	+19.97	+16.19	+
52	<i>Cronobacter</i>	<i>turicensis</i>	DSMZ 18703	/	4	+20.00	+16.75	+

*:1/10 dilution

INCLUSIVITY (ADRIA Développement, 2020)																									
Strains					iQ-Check <i>Cronobacter</i> spp. method - (37°C) BPW + specific supplement (d¼). Incubation for 18-24 h at 37°C ± 1°C																				
No	Genus	Species	N°	Origin	Inoculation level (CFU/ 225ml BPW)	PCR - Easy protocol												Confirmation		PCR on colonies					
						APF Classic			APF Fast			FDRS-APF Classic			FDRS-APF Fast			RAPID [®] Sakazakii Agar (24 h ± 2 h at 44°C ± 1°C)	Api ID 32E	APF Classic			APF Fast		
						Cq	I.C. Cq	Result	Cq	I.C. Cq	Result	Cq	I.C. Cq	Result	Cq	I.C. Cq	Result			Cq	I.C. Cq	Result	Cq	I.C. Cq	Result
1	<i>Cronobacter</i>	<i>dublinensis</i>	DSM18705	Dairy Product	12	21.35	31.72	+	20.46	32.08	+	22.18	31.99	+	22.83	33.76	+	+	+	20.6	34.12	+	21.69	34.8	+
2	<i>Cronobacter</i>	<i>malonaticus</i>	DSM18702	Dairy Product	2	21.48	31.6	+	20.94	32.47	+	19.34	32.87	+	20.93	33.74	+	+	+	21.24	32.14	+	21.04	32.34	+
3	<i>Cronobacter</i>	<i>malonaticus</i>	Ad1708	Dairy Product	21	19.10	34.21	+	19.14	35.65	+	19.06	34.83	+	18.71	35.63	+	+	+	20.27	33.22	+	20.4	33.93	+
4	<i>Cronobacter</i>	<i>muytjensii</i>	CIP103581	/	6	20.11	33.77	+	18.86	35.63	+	20.45	35.59	+	20.47	-	+	+	+	16.16	-	+	18.19	-	+
5	<i>Cronobacter</i>	<i>sakazakii</i>	Ad939	Infant formula	14	18.76	32.33	+	18.44	32.04	+	23.69	35.4	+	22.73	37.69	+	+	+	19.86	32.39	+	19.86	33.67	+
6	<i>Cronobacter</i>	<i>sakazakii</i>	Ad940	Infant formula	53	18.75	33.88	+	19.36	34.28	+	-/21.54*	-/33.52*	i/+*	23.93	-	+	+	+	18.66	-	+	19.33	39.99	+
7	<i>Cronobacter</i>	<i>sakazakii</i>	Ad941	Infant formula	22	18.18	34.89	+	19.55	33.71	+	22.99	-	+	20.85	-	+	+	+	18.18	-	+	19.47	40.56	+
8	<i>Cronobacter</i>	<i>sakazakii</i>	Ad942	Infant formula	27	18.38	35.36	+	19.95	34.52	+	-/21.24*	-/33.34*	i/+*	20.13	-	+	+	+	20.27	33.08	+	20.58	35.04	+
9	<i>Cronobacter</i>	<i>sakazakii</i>	Ad943	Infant formula	10	19.25	32.02	+	20.02	32.79	+	19.73	32.09	+	22.56	36.5	+	+	+	25.76	31.62	+	16.27	31.71	+
10	<i>Cronobacter</i>	<i>sakazakii</i>	Ad944	Infant formula	20	18.71	34.11	+	18.76	39.87	+	19.7	34.74	+	19.47	-	+	+	+	21.6	33.17	+	21.75	34.09	+
11	<i>Cronobacter</i>	<i>sakazakii</i>	Ad945	Infant formula	14	19.45	32.37	+	19.27	33.04	+	19.62	32.83	+	18.21	33.79	+	+	+	19.36	33.85	+	26.09	-	+
12	<i>Cronobacter</i>	<i>sakazakii</i>	Ad946	Infant formula	14	19.44	34.22	+	18.44	36.61	+	19.45	33.92	+	18.63	39.16	+	+	+	20.15	44.01	+	21.45	36.75	+
13	<i>Cronobacter</i>	<i>sakazakii</i>	Ad947	Infant formula	20	19.09	31.68	+	18.47	32.13	+	21.15	33.54	+	18.35	32.65	+	+	+	19.91	32.13	+	28.7	35.81	+
14	<i>Cronobacter</i>	<i>sakazakii</i>	Ad948	Infant formula	24	20.00	32.25	+	19.26	36.89	+	25.3	35.08	+	19.83	32.74	+	+	+	19.19	33.5	+	22.99	31.7	+
15	<i>Cronobacter</i>	<i>sakazakii</i>	Ad949	Infant formula	8	18.40	32.02	+	19.14	32.17	+	22.71	34.6	+	17.96	32.35	+	+	+	23.35	36.44	+	24.34	33.12	+
16	<i>Cronobacter</i>	<i>sakazakii</i>	Ad950	Infant formula	19	18.70	32.19	+	19.13	31.78	+	24.12	35.3	+	20.87	32.86	+	+	+	23.31	31.52	+	23.78	31.54	+
17	<i>Cronobacter</i>	<i>sakazakii</i>	Ad951	Infant formula	14	18.45	34.83	+	19.14	33.16	+	19.45	35.04	+	21.11	-	+	+	+	21.7	-	+	23.63	32.53	+
18	<i>Cronobacter</i>	<i>sakazakii</i>	Ad952	Infant formula	24	19.69	32.02	+	19.35	31.69	+	19.72	33	+	18.26	33.96	+	+	+	19.5	32.84	+	18.65	35.28	+
19	<i>Cronobacter</i>	<i>sakazakii</i>	Ad953	Infant formula	32	19.54	33.3	+	18.82	36.04	+	20.04	34.54	+	19.5	35.83	+	+	+	21.48	32.93	+	22.8	32.69	+
20	<i>Cronobacter</i>	<i>sakazakii</i>	Ad963	Infant formula	19	20.50	32.81	+	19.07	35.73	+	19.97	33.58	+	18.86	38.41	+	+	+	20.75	36.17	+	21.72	42.42	+
21	<i>Cronobacter</i>	<i>sakazakii</i>	95	Infant formula	21	19.54	32.41	+	19.07	33.07	+	19.52	32.4	+	18.95	32.82	+	+	+	22.38	32.05	+	21.63	32.27	+
22	<i>Cronobacter</i>	<i>sakazakii</i>	Ad704	Infant formula	26	19.58	32.01	+	18.77	32.15	+	22.93	32.18	+	20.9	32.27	+	+	+	17.63	-	+	20.38	33.97	+
23	<i>Cronobacter</i>	<i>sakazakii</i>	Ad831	Infant formula	26	18.08	32.12	+	19.2	32.23	+	19.45	31.98	+	18.69	32.55	+	+	+	20.84	32.57	+	20.49	33.96	+
24	<i>Cronobacter</i>	<i>sakazakii</i>	Ad829	Infant formula	16	18.08	35.57	+	18.76	35.37	+	22.33	41.44	+	22.07	41.03	+	+	+	19.48	34.63	+	19.56	38.28	+
25	<i>Cronobacter</i>	<i>sakazakii</i>	Ad916	Infant formula	15	19.52	32.74	+	20.2	32.92	+	23.43	33.35	+	22.1	32.24	+	+	+	19.19	-	+	21.05	33.09	+
26	<i>Cronobacter</i>	<i>sakazakii</i>	Ad893	Infant formula	17	20.91	31.82	+	18.55	31.99	+	19.9	32.17	+	18.65	32.35	+	+	+	19.7	34.13	+	20.1	33.13	+
27	<i>Cronobacter</i>	<i>sakazakii</i>	Ad894	Infant formula	14	19.89	33.38	+	18.75	35.25	+	22.89	36.86	+	19.49	34.08	+	+	+	19.38	34.67	+	19.95	36.22	+
28	<i>Cronobacter</i>	<i>sakazakii</i>	Ad895	Infant formula	7	18.84	33.7	+	18.53	35.88	+	19.32	34.48	+	18.07	40.62	+	+	+	19.02	-	+	18.35	-	+
29	<i>Cronobacter</i>	<i>sakazakii</i>	Ad896	Infant formula	13	22.05	31.89	+	19.16	32.21	+	20.46	32.1	+	19.14	32.53	+	+	+	19.87	32.58	+	19.51	35.85	+
30	<i>Cronobacter</i>	<i>sakazakii</i>	Ad897	Infant formula	21	21.55	32.27	+	18.48	36.85	+	20.46	36.47	+	19.11	40.68	+	+	+	19.44	36.76	+	19.58	36.79	+
31	<i>Cronobacter</i>	<i>sakazakii</i>	Ad898	Infant formula	9	21.32	32.12	+	19.37	32.87	+	19.87	33.23	+	19.16	34.16	+	+	+	19.97	32.88	+	20.22	33.94	+
32	<i>Cronobacter</i>	<i>dublinensis lactaridi</i>	DSMZ18707 T	Dairy Product	12	22.48	31.36	+	20.3	32.27	+	20.2	32.46	+	20.15	32.41	+	+	+	21.21	32.2	+	21.49	31.9	+
33	<i>Cronobacter</i>	<i>dublinensis lausannensis</i>	DSMZ 18706 T	Dairy Product	23	21.19	31.82	+	20.43	32.32	+	20.4	32.17	+	25.33	36.34	+	+	+	21.52	33.1	+	19.76	36.52	+
34	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1418	Infant formula	17	20.07	31.95	+	18.9	32.36	+	19.68	32.19	+	20.52	33.49	+	+	+	21.24	32.03	+	21.28	32.29	+
35	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1419	Infant formula	24	21.01	32.01	+	18.26	32.58	+	22.09	31.93	+	21.24	32.57	+	+	+	20.41	31.66	+	21.03	32.08	+
36	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1420	Infant formula	14	20.22	31.61	+	18.58	32.18	+	19.06	32.06	+	18.84	32.7	+	+	+	18.49	37.31	+	18.12	38.45	+
37	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1421	Infant formula	12	20.37	32.08	+	18.16	32.16	+	21.5	32.16	+	20.44	31.83	+	+	+	20.14	33.01	+	20.05	32.95	+

INCLUSIVITY (ADRIA Développement, 2020)

iQ-Check <i>Cronobacter</i> spp. method - (37°C) BPW + specific supplement (d¼). Incubation for 18-24 h at 37°C ± 1°C																									
Strains						PCR - Easy protocol																			
No	Genus	Species	N°	Origin	Inoculation level (CFU/ 225ml BPW)	APF Classic			APF Fast			FDRS-APF Classic			FDRS-APF Fast			Confirmation		PCR on colonies					
						Cq	I.C. Cq	Result	Cq	I.C. Cq	Result	Cq	I.C. Cq	Result	Cq	I.C. Cq	Result	RAPID'Sakazakii Agar (24 h ± 2 h at 44°C ± 1°C)	Api ID 32E	APF Classic			APF Fast		
																				Cq	I.C. Cq	Result	Cq	I.C. Cq	Result
38	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1424	Infant formula	16	30.21	32.2	+	18.35	33.36	+	24.2	34.38	+	21.08	32.12	+	+	+	18.36	38.13	+	18.22	41.11	+
39	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1425	Infant formula	11	18.82	34.56	+	18.89	35.62	+	22.36	46.66	+	18.56	39.54	+	+	+	20.01	33.8	+	19.58	40.55	+
40	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1426	Infant formula	26	18.26	32.21	+	19.28	32.05	+	19.92	32.7	+	21.09	34.02	+	+	+	20.8	32.04	+	21.27	31.48	+
41	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1427	Infant formula	33	17.51	36.94	+	18.71	34.9	+	19.28	34.49	+	24.32	-	+	+	+	16.89	-	+	17.35	-	+
42	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1428	Infant formula	13	18.78	34.42	+	19.22	34.3	+	19.14	34.66	+	22.88	-	+	+	+	23.5	32.41	+	23.4	32.04	+
43	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1429	Infant formula	12	20.89	31.63	+	19.07	32.23	+	20.58	31.95	+	20.43	32.25	+	+	+	22.11	31.99	+	22.31	31.47	+
44	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1430	Infant formula	27	18.37	32.89	+	18.31	32.97	+	21.67	32.21	+	21.49	32.18	+	+	+	21.19	31.81	+	21.27	31.62	+
45	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1431	Infant formula	21	18.10	32.06	+	18.27	32.28	+	18.45	32.14	+	19.11	33.45	+	+	+	20.21	31.78	+	20.96	32.38	+
46	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1432	Infant formula	16	18.56	34.64	+	19.11	34.5	+	21.13	42.23	+	20.27	-	+	+	+	20.92	33.28	+	21.14	34.21	+
47	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1433	Infant formula	19	18.41	34.84	+	18.08	33.8	+	19.73	33.16	+	20.08	37.17	+	+	+	21.53	32.76	+	20.05	34.98	+
48	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1434	Infant formula	18	18.03	35.23	+	19.09	34.26	+	25.03	37.99	+	23.3	34.78	+	+	+	22.51	32.72	+	20.24	36.59	+
49	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1435	Infant formula	15	20.36	35.41	+	19.44	36.32	+	22.54	34.65	+	23.15	36.13	+	+	+	23.18	34.1	+	19.61	-	+
50	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1436	Infant formula	9	20.64	33.05	+	19.28	34.84	+	21.95	32.64	+	22.54	33.44	+	+	+	19.37	34.79	+	19.55	34.32	+
51	<i>Cronobacter</i>	<i>turicensis</i>	Ad 1445	Infant formula	20	31.19	31.29	+	19.62	32.16	+	22.49	32.12	+	21.11	32.01	+	+	+	20.98	31.21	+	20.26	33.46	+
52	<i>Cronobacter</i>	<i>turicensis</i>	DSMZ 18703	/	20	20.92	31.61	+	19.58	32.32	+	20.18	32.25	+	19.31	32.33	+	+	+	19.29	33.45	+	20.45	32.48	+

EXCLUSIVITY (Initial validation, 2007)						
	Genus	Species	N°	Origin	Inoculation level CFU/ml BPW	PCR Easy Protocol BPW 16h 37°C
1.	<i>Citrobacter</i>	<i>braakii</i>	Ad833	Beef	4.00E+05	-
2.	<i>Citrobacter</i>	<i>diversus</i>	Ad173	Dairy product	2.60E+05	-
3.	<i>Citrobacter</i>	<i>fameri</i>	Ad116	Environmental sample	3.50E+05	-
4.	<i>Citrobacter</i>	<i>freundii</i>	39	Environmental sample	5.10E+05	-
5.	<i>Citrobacter</i>	<i>koseri</i>	CIP105177	/	3.40E+05	-
6.	<i>Enterobacter</i>	<i>aerogenes</i>	Ad889	Meat flour	2.90E+05	-
7.	<i>Enterobacter</i>	<i>agglomerans</i>	11	Dairy product	2.60E+05	-
8.	<i>Enterobacter</i>	<i>agglomerans</i>	136	Dairy product	1.50E+05	-
9.	<i>Enterobacter</i>	<i>amnigenus</i>	52	Vegetables	1.10E+05	-
10.	<i>Enterobacter</i>	<i>amnigenus</i>	129	Raw milk	1.00E+05	-
11.	<i>Enterobacter</i>	<i>amnigenus</i>	A00C068	Poultry	1.70E+05	-
12.	<i>Enterobacter</i>	<i>cloacae</i>	51	Vegetables	4.80E+05	-
13.	<i>Enterobacter</i>	<i>cloacae</i>	10	Dairy product	1.80E+05	-
14.	<i>Enterobacter</i>	<i>fergusonii</i>	2876	Environmental sample	4.00E+05	-
15.	<i>Enterobacter</i>	<i>gergoviae</i>	CIP76.1	/	3.30E+05	-
16.	<i>Enterobacter</i>	<i>helveticus</i>	DSM 18396 T	fruit powder	1.40E+05	-
17.	<i>Enterobacter</i>	<i>hormaechei</i>	Ad990	Butter	4.40E+05	-
18.	<i>Enterobacter</i>	<i>intermedius</i>	60	Vegetables	1.40E+05	-
19.	<i>Enterobacter</i>	<i>kobei</i>	Ad706	Milk powder	2.60E+05	-
20.	<i>Escherichia</i>	<i>coli</i>	16	Dairy product	3.80E+05	-
21.	<i>Escherichia</i>	<i>hermanii</i>	Ad462	Dairy product	2.00E+05	-
22.	<i>Hafnia</i>	<i>alvei</i>	Ad245	Dairy product	4.20E+05	-
23.	<i>Klebsiella</i>	<i>pneumoniae</i>	122	Dairy product	3.10E+05	-
24.	<i>Kluyvera</i>	<i>oxytoca</i>	MI030497b	Milk powder	2.70E+05	-
25.	<i>Leclercia</i>	<i>adecarboxylata</i>	Ad707	Milk powder	8.60E+04	-
26.	<i>Salmonella</i>	<i>arizonae</i> (51:z4,z23)	CIP 5523	/	1.40E+05	-
27.	<i>Salmonella</i>	<i>diarizonae</i> (65 :c :z)	Ad 1298	Dairy environmental sample	4.80E+05	-
28.	<i>Salmonella</i>	Typhimurium	Ad1333	Dairy product	4.60E+05	-
29.	<i>Serratia</i>	<i>ficaria</i>	113	Vegetables	4.20E+05	-
30.	<i>Serratia</i>	<i>marcescens</i>	Ad455	Raw milk	4.20E+05	-
31.	<i>Yersinia</i>	<i>intermediae</i>	Ad133	Dairy product	4.70E+05	-

Appendix 7 – Inter-laboratory study: results obtained by the collaborative laboratories and the expert laboratory

Laboratory **A**

Aerobic mesophilic flora: 66/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID'Sakazakii		
A2	-	/	/	/	-	-	/	-	NA
A6	-	/	/	/	-	-	/	-	NA
A10	-	/	/	/	-	-	/	-	NA
A11	-	/	/	/	-	-	/	-	NA
A17	-	/	/	/	-	+ (CT 29,59)	-	-	PD _{FP(alt)}
A19	-	/	/	/	-	-	/	-	NA
A22	-	/	/	/	-	-	/	-	NA
A24	-	/	/	/	-	-	/	-	NA
A1	+	+	-	+	+	-	/	-	ND
A4	-	/	/	/	-	+ (CT 41,72)	-	-	PD _{FP(alt)}
A9	-	/	/	/	-	+	+	+	PD
A12	-	/	/	/	-	+	+	+	PD
A14	-	/	/	/	-	+	+	+	PD
A18	-	/	/	/	-	+	+	+	PD
A20	-	/	/	/	-	+	+	+	PD
A21	-	/	/	/	-	-	/	-	NA
A3	+	+	-	+	+	+	+	+	PA
A5	+	+	-	+	+	+	+	+	PA
A7	+	+	-	+	+	+	+	+	PA
A8	+	+	-	+	+	+	+	+	PA
A13	+	+	-	+	+	+	+	+	PA
A15	+	+	-	+	+	+	+	+	PA
A16	+	+	-	+	+	+	+	+	PA
A23	+	+	-	+	+	+	+	+	PA

Laboratory B
Aerobic mesophilic flora: 44/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID'Sakazakii		
B2	-	/	/	/	-	-	/	-	NA
B6	-	/	/	/	-	-	/	-	NA
B10	-	/	/	/	-	+(CT 46,4)	-	-	PD _{FP(alt)}
B11	-	/	/	/	-	-	/	-	NA
B17	-	/	/	/	-	-	/	-	NA
B19	-	/	/	/	-	-	/	-	NA
B22	-	/	/	/	-	-	/	-	NA
B24	-	/	/	/	-	-	/	-	NA
B1	-	/	/	/	-	+	+	+	PD
B4	-	/	/	/	-	+	+	+	PD
B9	-	/	/	/	-	+	+	+	PD
B12	-	/	/	/	-	+	+	+	PD
B14	-	/	/	/	-	+	+	+	PD
B18	-	/	/	/	-	-	/	-	NA
B20	+	+	-	+	+	-	/	-	ND
B21	+	+	-	+	+	+	+	+	PA
B3	+	+	-	+	+	+	+	+	PA
B5	+	+	-	+	+	+	+	+	PA
B7	+	+	-	+	+	+	+	+	PA
B8	+	+	-	+	+	+	+	+	PA
B13	+	+	-	+	+	+	+	+	PA
B15	+	+	-	+	+	+	+	+	PA
B16	+	+	-	+	+	+	+	+	PA
B23	+	+	-	+	+	+	+	+	PA

Laboratory **C**
 Aerobic mesophilic flora:46 cfu/ml

Reference method : micropipette was used to transfer 0.1 ml BPW into mLST+V

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID'Sakazakii		
C2	-	/	/	/	-	-	/	-	NA
C6	+	+	-	+	+	+	+	+	PA
C10	-	/	/	/	-	+	+	+	PD
C11	-	/	/	/	-	+	+	+	PD
C17	-	/	/	/	-	+	+	+	PD
C19	-	/	/	/	-	+	+	+	PD
C22	+	+	-	+	+	+	+	+	PA
C24	-	/	/	/	-	+	-	-	PD FP(alt)
C1	+	+	-	+	+	+	-	-	PA FP(alt)
C4	+	+	-	+	+	+	+	+	PA
C9	+	+	-	+	+	-	/	-	ND
C12	+	+	-	+	+	+	+	+	PA
C14	+	+	-	+	+	-	/	-	ND
C18	-	/	/	/	-	+	+	+	PD
C20	-	/	/	/	-	-	/	-	NA
C21	-	/	/	/	-	+	+	+	PD
C3	+	+	-	+	+	+	+	+	PA
C5	+	+	-	+	+	+	+	+	PA
C7	+	+	-	+	+	+	+	+	PA
C8	+	+	-	+	+	+	+	+	PA
C13	+	+	-	+	+	+	+	+	PA
C15	+	+	-	+	+	+	+	+	PA
C16	+	+	-	+	+	+	+	+	PA
C23	+	+	-	+	+	+	+	+	PA

Laboratory **D**
 Aerobic mesophilic flora:50 cfu/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
D2	-	/	/	/	-	-	/	-	NA
D6	-	/	/	/	-	-	/	-	NA
D10	-	/	/	/	-	+(CT29,4)	/	-	PD FP(alt)
D11	-	/	/	/	-	+(CT35,9)	/	-	PD FP(alt)
D17	-	/	/	/	-	+(CT42,51)	/	-	PD FP(alt)
D19	-	/	/	/	-	-	/	-	NA
D22	-	/	/	/	-	+(CT39,75)	/	-	PD FP(alt)
D24	-	/	/	/	-	+(CT34,49)	/	-	PD FP(alt)
D1	+	+	-	+	+	+	+	+	PA
D4	-	/	/	/	-	+	+	+	PD
D9	+	+	-	+	+	-	-	-	ND
D12	+	+	-	+	+	+	+	+	PA
D14	+	+	-	+	+	+	+	+	PA
D18	-	/	/	/	-	-	/	-	NA
D20	-	/	/	/	-	-	/	-	NA
D21	-	/	/	/	-	+	+	+	PD
D3	+	+	-	+	+	+	+	+	PA
D5	+	+	-	+	+	+	+	+	PA
D7	+	+	-	+	+	+	+	+	PA
D8	+	+	-	+	+	+	+	+	PA
D13	+	+	-	+	+	+	+	+	PA
D15	+	+	-	+	+	+	+	+	PA
D16	+	+	-	+	+	+	+	+	PA
D23	+	+	-	+	+	+	+	+	PA

Laboratory **E**

Aerobic mesophilic flora: 33cfu/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID'Sakazakii		
E2	-	/	/	/	-	-	/	-	NA
E6	-	/	/	/	-	-	/	-	NA
E10	-	/	/	/	-	-	/	-	NA
E11	-	/	/	/	-	-	/	-	NA
E17	-	/	/	/	-	-	/	-	NA
E19	-	/	/	/	-	-	/	-	NA
E22	-	/	/	/	-	-	/	-	NA
E24	-	/	/	/	-	-	/	-	NA
E1	+	+	-	+	+	+	+	+	PA
E4	-	/	/	/	-	+	+	+	PD
E9	+	+	-	+	+	+	+	+	PA
E12	+	+	-	+	+	+	+	+	PA
E14	+	+	-	+	+	+	+	+	PA
E18	+	+	-	+	+	-	/	-	ND
E20	+	+	-	+	+	-	/	-	ND
E21	-	/	/	/	-	-	/	-	NA
E3	+	+	-	+	+	+	+	+	PA
E5	+	+	-	+	+	+	+	+	PA
E7	+	+	-	+	+	+	+	+	PA
E8	+	+	-	+	+	+	+	+	PA
E13	+	+	-	+	+	+	+	+	PA
E15	+	+	-	+	+	+	+	+	PA
E16	+	+	-	+	+	+	+	+	PA
E23	+	+	-	+	+	+	+	+	PA

Laboratory **F**
 Aerobic mesophilic flora:37 cfu/ml

Reference method : micropipette was used to transfer 0.1 ml BPW into mLST+V

N°Sample	Reference method ISO / TS 22964				Alternative method: iQ-Check <i>Cronobacter</i> spp				Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
F2	-	/	/	/	-	-	/	-	NA
F6	-	/	/	/	-	-	/	-	NA
F10	-	/	/	/	-	-	/	-	NA
F11	-	/	/	/	-	-	/	-	NA
F17	-	/	/	/	-	+(CT34,31)	-	-	PD ^{FP(alt)}
F19	-	/	/	/	-	-	/	-	NA
F22	-	/	/	/	-	-	/	-	NA
F24	+	+	-	+	+	-	/	-	ND
F1	+	+	-	+	+	-	/	-	ND
F4	+	+	-	+	+	-	/	-	ND
F9	+	+	-	+	+	+	+	+	PA
F12	-	/	/	/	-	+	+	+	PD
F14	+	+	-	+	+	-	/	-	ND
F18	+	+	-	+	+	+	+	+	PA
F20	+	+	-	+	+	+	+	+	PA
F21	-	/	/	/	-	-	/	-	NA
F3	+	+	-	+	+	+	+	+	PA
F5	+	+	-	+	+	+	+	+	PA
F7	+	+	-	+	+	+	+	+	PA
F8	+	+	-	+	+	+	+	+	PA
F13	+	+	-	+	+	+	+	+	PA
F15	+	+	-	+	+	+	+	+	PA
F16	+	+	-	+	+	+	+	+	PA
F23	+	+	-	+	+	+	+	+	PA

Laboratory **G**
 Aerobic mesophilic flora:50(Ne)cfu/ml

Reference method : micropipette was used to transfer 0.1 ml BPW into mLST+V

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
G2	-	/	/	/	-	-	-	-	NA
G6	-	/	/	/	-	-	-	-	NA
G10	-	/	/	/	-	-	-	-	NA
G11	-	/	/	/	-	-	-	-	NA
G17	+	+	-	+	+	-	-	-	ND
G19	-	/	/	/	-	-	-	-	NA
G22	+	+	-	+	+	-	-	-	ND
G24	-	/	/	/	-	-	-	-	NA
G1	-	/	/	/	-	+	+	+	PD
G4	+	+	-	+	+	+	+	+	PA
G9	-	/	/	/	-	+	+	+	PD
G12	-	/	/	/	-	-	-	-	NA
G14	+	+	-	+	+	+	+	+	PA
G18	+	+	-	+	+	+	+	+	PA
G20	+	+	-	+	+	+	+	+	PA
G21	-	/	/	/	-	-	-	-	NA
G3	+	+	-	+	+	+	+	+	PA
G5	+	+	-	+	+	+	+	+	PA
G7	+	+	-	+	+	+	+	+	PA
G8	+	+	-	+	+	+	+	+	PA
G13	+	+	-	+	+	+	+	+	PA
G15	+	+	-	+	+	+	+	+	PA
G16	+	+	-	+	+	+	+	+	PA
G23	+	+	-	+	+	+	+	+	PA

Laboratory **H**
 Aerobic mesophilic flora:48 cfu/ml

Reference method : micropipette was used to transfer 0.1 ml into mLST+V

N°Sample	Reference method ISO / TS 22964				Alternative method: iQ-Check <i>Cronobacter</i> spp				Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
H2	-	/	/	/	-	-	-	-	NA
H6	+	+	-	+	+	-	-	-	ND
H10	-	/	/	/	-	+(CT41,94)	-	-	PD FP(alt)
H11	+	+	-	+	+	-	-	-	ND
H17	-	/	/	/	-	+(CT27,30)	-	-	PD FP(alt)
H19	+	+	-	+	+	-	-	-	ND
H22	+1 col	+	-	+	+	+(CT43,13)	-	-	PD FP(alt)
H24	-	/	/	/	-	-	-	-	NA
H1	+	+	-	+	+	+	+	+	PA
H4	-	/	/	/	-	+	+	+	PD
H9	-	/	/	/	-	-	-	-	NA
H12	+	+	-	+	+	+	+	+	PA
H14	+	+	-	+	+	+	+	+	PA
H18	+	+	-	+	+	-	-	-	ND
H20	+	+	-	+	+	+	+	+	PA
H21	+	+	-	+	+	+	+	+	PA
H3	+	+	-	+	+	+	+	+	PA
H5	+	+	-	+	+	+	+	+	PA
H7	+	+	-	+	+	+	+	+	PA
H8	+	+	-	+	+	+	+	+	PA
H13	+	+	-	+	+	+	+	+	PA
H15	+	+	-	+	+	+	+	+	PA
H16	+	+	-	+	+	+	+	+	PA
H23	+	+	-	+	+	+	+	+	PA

Laboratory I
Aerobic mesophilic flora: 43 cfu/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
I2	-	/	/	/	-	-	/	-	NA
I6	-	/	/	/	-	-	/	-	NA
I10	-	/	/	/	-	-	/	-	NA
I11	-	/	/	/	-	-	/	-	NA
I17	-	/	/	/	-	-	/	-	NA
I19	-	/	/	/	-	-	/	-	NA
I22	-	/	/	/	-	-	/	-	NA
I24	-	/	/	/	-	-	/	-	NA
I1	+	+	-	+	+	-	/	-	ND
I4	-	/	/	/	-	-	/	-	NA
I9	+	+	-	+	+	-	/	-	ND
I12	-	/	/	/	-	-	/	-	NA
I14	+	+	-	+	+	+	+	+	PA
I18	+	+	-	+	+	+	+	+	PA
I20	-	/	/	/	-	+	+	+	PD
I21	+	+	-	+	+	-	/	-	ND
I3	+	+	-	+	+	+	+	+	PA
I5	+	+	-	+	+	+	+	+	PA
I7	+	+	-	+	+	+	+	+	PA
I8	+	+	-	+	+	+	+	+	PA
I13	+	+	-	+	+	+	+	+	PA
I15	+	+	-	+	+	+	+	+	PA
I16	+	+	-	+	+	+	+	+	PA
I23	+	+	-	+	+	+	+	+	PA

Laboratory J

Aerobic mesophilic flora: 8 cfu/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
J2	-	/	/	/	-	-	-	-	NA
J6	-	/	/	/	-	-	-	-	NA
J10	-	/	/	/	-	-	-	-	NA
J11	-	/	/	/	-	-	-	-	NA
J17	-	/	/	/	-	-	-	-	NA
J19	-	/	/	/	-	-	-	-	NA
J22	-	/	/	/	-	-	-	-	NA
J24	-	/	/	/	-	-	-	-	NA
J1	-	/	/	/	-	-	-	-	NA
J4	+	+	-	+	+	+	+	+	PA
J9	+	+	-	+	+	+	+	+	PA
J12	+	+	-	+	+	+	+	+	PA
J14	+	+	-	+	+	+	+	+	PA
J18	+	+	-	+	+	-	-	-	ND
J20	+	+	-	+	+	-	-	-	ND
J21	-	/	/	/	-	-	-	-	NA
J3	+	+	-	+	+	+	+	+	PA
J5	+	+	-	+	+	+	+	+	PA
J7	+	+	-	+	+	+	+	+	PA
J8	+	+	-	+	+	+	+	+	PA
J13	+	+	-	+	+	+	+	+	PA
J15	+	+	-	+	+	+	+	+	PA
J16	+	+	-	+	+	+	+	+	PA
J23	+	+	-	+	+	+	+	+	PA

Laboratory **K**
 Aerobic mesophilic flora: 2,0 E10+05 cfu/ml

N°Sample	Reference method ISO / TS 22964				Alternative method: iQ-Check <i>Cronobacter</i> spp				Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
K2	-	/	/	/	-	-	-	-	NA
K6	-	/	/	/	-	-	-	-	NA
K10	-	/	/	/	-	-	-	-	NA
K11	-	/	/	/	-	-	-	-	NA
K17	-	/	/	/	-	-	-	-	NA
K19	-	/	/	/	-	-	-	-	NA
K22	-	/	/	/	-	-	-	-	NA
K24	-	/	/	/	-	-	-	-	NA
K1	-	/	/	/	-	+	+	+	PD
K4	-	/	/	/	-	-	-	-	NA
K9	-	/	/	/	-	-	-	-	NA
K12	+	+	-	+	+	+	+	+	PA
K14	-	/	/	/	-	+	+	+	PD
K18	+	+	-	+	+	-	-	-	ND
K20	-	/	/	/	-	+	+	+	PD
K21	+	+	-	+	+	+	+	+	PA
K3	+	+	-	+	+	+	+	+	PA
K5	+	+	-	+	+	+	+	+	PA
K7	+	+	-	+	+	+	+	+	PA
K8	+	+	-	+	+	+	+	+	PA
K13	+	+	-	+	+	+	+	+	PA
K15	+	+	-	+	+	+	+	+	PA
K16	+	+	-	+	+	+	+	+	PA
K23	+	+	-	+	+	+	+	+	PA

Laboratory L
 Aerobic mesophilic flora: 43/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID' <i>Sakazakii</i>		
L2	-	/	/	/	-	-	-	-	NA
L6	-	/	/	/	-	-	-	-	NA
L10	-	/	/	/	-	-	-	-	NA
L11	-	/	/	/	-	-	-	-	NA
L17	-	/	/	/	-	-	-	-	NA
L19	-	/	/	/	-	-	-	-	NA
L22	-	/	/	/	-	-	-	-	NA
L24	-	/	/	/	-	-	-	-	NA
L1	-	/	/	/	-	-	-	-	NA
L4	-	/	/	/	-	+	+	+	PD
L9	-	/	/	/	-	-	-	-	NA
L12	+	+	-	+	+	-	-	-	ND
L14	+	+	-	+	+	-	-	-	ND
L18	-	/	/	/	-	-	-	-	NA
L20	+	+	-	+	+	+	+	+	PA
L21	-	-	-	-	-	-	-	-	NA
L3	+	+	-	+	+	+	+	+	PA
L5	+	+	-	+	+	+	+	+	PA
L7	+	+	-	+	+	+	+	+	PA
L8	+	+	-	+	+	+	+	+	PA
L13	+	+	-	+	+	+	+	+	PA
L15	+	+	-	+	+	+	+	+	PA
L16	+	+	-	+	+	+	+	+	PA
L23	+	+	-	+	+	+	+	+	PA

Laboratory **M**
 Aerobic mesophilic flora: 50/ml

N°Sample	Reference method ISO / TS 22964					Alternative method: iQ-Check <i>Cronobacter</i> spp			Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID'Sakazakii		
M2	-	/	/	/	-	-	/	-	NA
M6	-	/	/	/	-	+	+	+	PD
M10	-	/	/	/	-	+(CT 44,92)	-	-	PD FP(alt)
M11	-	/	/	/	-	-	/	-	NA
M17	-	/	/	/	-	+	+	+	PD
M19	-	/	/	/	-	+(CT 39,07)	-	-	PD FP(alt)
M22	-	/	/	/	-	+	+	+	PD
M24	-	/	/	/	-	-	/	-	NA
M1	-	/	/	/	-	+	+	+	PD
M4	-	/	/	/	-	+	+	+	PD
M9	-	/	/	/	-	+(CT 33.51)	-	-	PD FP(alt)
M12	+	+	-	+	+	-	/	-	ND
M14	+	+	-	+	+	+	+	+	PA
M18	+	+	-	+	+	-	/	-	ND
M20	-	/	/	/	-	+	+	+	PD
M21	-	/	/	/	-	+(CT 28.42)	-	-	PD FP(alt)
M3	+	+	-	+	+	+	+	+	PA
M5	+	+	-	+	+	+	+	+	PA
M7	+	+	-	+	+	+	+	+	PA
M8	+	+	-	+	+	+	+	+	PA
M13	+	+	-	+	+	+	+	+	PA
M15	+	+	-	+	+	+	+	+	PA
M16	+	+	-	+	+	-	/	-	ND
M23	+	+	-	+	+	+	+	+	PA

Laboratory N (ADRIA)

Aerobic mesophilic flora:145cfu/ml

N°Sample	Reference method ISO / TS 22964♦				Alternative method: iQ-Check <i>Cronobacter</i> spp				Agreement
	Typical colonies		Confirmation		Final result	PCR Result	Confirmation	Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			RAPID'Sakazakii		
N2	-	/	/	/	-	-	-	-	NA
N6	-	/	/	/	-	-	-	-	NA
N10	-	/	/	/	-	i/-	-	-	NA
N11	-	/	/	/	-	-	-	-	NA
N17	-	/	/	/	-	-	-	-	NA
N19	-	/	/	/	-	-	-	-	NA
N22	-	/	/	/	-	-	-	-	NA
N24	-	/	/	/	-	-	-	-	NA
N1	+	+	-	+	+	+(CT40.67)	-	-	PA _{FP(alt)}
N4	+	+	-	+	+	-	-	-	ND
N9	-	/	/	/	-	+	+	+	PD
N12	+	+	-	+	+	+	+	+	PA
N14	-	/	/	/	-	+	+	+	PD
N18	-	/	/	/	-	-	-	-	NA
N20	+	+	-	+	+	-	-	-	ND
N21	-	/	/	/	-	+	+	+	PD
N3	+	+	-	+	+	+	+	+	PA
N5	+	+	-	+	+	+	+	+	PA
N7	+	+	-	+	+	+	+	+	PA
N8	+	+	-	+	+	+	+	+	PA
N13	+	+	-	+	+	+	+	+	PA
N15	+	+	-	+	+	+	+	+	PA
N16	+	+	-	+	+	+	+	+	PA
N23	+	+	-	+	+	+	+	+	PA

 ♦ Analyses performed according to the COFRAC accreditation (Accreditation Testing n°1-0144, scope available on www.cofrac.fr)