

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

Validation study according to the EN ISO 16140-2:2016

RAPID'Sakazakii

(Certificate number: BKR 07/22 - 05/12)

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

Qualitative method

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This report consists of 74 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♦.

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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 EN ISO 16140-2:2016 and the AFNOR technical rules (PR Revision 7).

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): 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 (PR Revision 7).
Reference methods[♦]	ISO 22964 (April 2017) - Microbiology of the food chain - Horizontal method for the detection of <i>Cronobacter</i> spp.
Alternative method	RAPID'Sakazakii
Scope	Infant formula and infant cereals with or without probiotics including ingredients (30 g and 375 g test portions)
Certification organism	AFNOR Certification (http://nf-validation.afnor.org/)

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

1 INTRODUCTION

The RAPID' *Sakazakii* was validated with the certificate number BRD 07/22 - 05/12 according to the ISO 16140 standard (2003) for infant formula (30 g sample size). The method was renewed in March 2016 according to the same version of the ISO 16140.

In April 2020, the method was renewed according to the ISO 16140-2:2016 and an extension study was performed for a 375 g sample size.

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.

Two expert laboratories were involved in this study:

- For the renewal study, the analyses were subcontracted to **Q-Laboratories Inc** (1930 Radcliff Drive, Cincinnati, OH 45204). The following parts were tested:
 - Sensitivity,
 - RLOD.

- For the extension study, the analyses were carried out by **ADRIA**. The following parts were tested:
 - Sensitivity,
 - RLOD,
 - Inclusivity.

The certificate was renewed in April 2024 according to the ISO16140-2:2016.

2 METHOD PROTOCOLS

2.1 Alternative method

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

2.1.1 Principle

The RAPID' *Sakazakii* method is based on an enzymatic activity characteristic of *Cronobacter* spp. Typical colonies appear blue to blue green. Sodium desoxycholate and cristal violet inhibits the background microflora.

The presumptive positive results are confirmed using the ISO 22964 confirmatory tests or by the iQ-Check *Cronobacter* PCR test.

2.1.2 Protocol

The different steps are described in **Table 1**.

Table 1 - Protocols

	Category 1: Infant formula and infant cereals with and without probiotics including ingredients (30 g sample size)	Category 2: Infant formula and infant cereals with and without probiotics including ingredients (375 g sample size)
Enrichment	30 g + 270 mL BPW* 18 h ± 2 h at 37°C ± 1°C	375 g + 1125 mL pre-warmed (37°C) BPW with PIF supplement* 18 to 24 h at 37°C ± 1°C
Streaking	10 µL onto RAPID'Sakazakii Incubation 24 h ± 2 h at 44°C ± 1°C	
Confirmation	1 to 5 colonies by the tests described in the ISO method (biochemical tests) with or without purification step on TSA or by PCR (iQ-Check <i>Cronobacter</i>) on isolated colony	
Enrichment broth storage	48 h at 5°C ± 3°C	72 h at 5°C ± 3°C
Plate storage	72 h at 5°C ± 3°C	

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

2.1.3 Restrictions

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

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

The modifications which occur in the version published in 2017 are considered as major with impacts on the performances; this required to perform again the sensitivity and the RLOD parts.

Note that, based on previous data, big sample sizes cannot be used in the reference method procedure due to the impact on the detection levels and the sensitivity performances. 10 g test portions were thus used for the reference method while 30 g or 375 g test portions were tested for the alternative method depending on the protocol tested.

The flow diagrams are 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.

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 diversity of samples and strains representative of agri-food products. This does not constitute an exhaustive list of the different matrices included in the scope.

For any comment on the alternative method, please contact AFNOR Certification at <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. 60 samples were tested for the renewal study (category 1) and 66 samples for the extension study (category 2). Taking into both categories, 126 samples were tested providing 71 positive and 55 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	
Qlab	1	Infant formula and infant cereals with and without probiotics including ingredients (30 g sample size)	a	11	9	20
			b	12	8	20
			c	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	13	9	22
			b	11	11	22
			c	10	12	22
		Total	34	32	66	
All categories			71	55	126	

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**.

58 samples were artificially contaminated, using 12 different strains. 55 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
All categories	16	0	3	0	0	33	14	0	71
%	22,5	0,0	4,2	0,0	0,0	53,5	19,7	0,0	100,0

The percentage of samples inoculated between 3 or 5 CFU and 10 CFU is 19.7 %. Note according to the AFNOR technical rules (Rev 6), the maximum allowed is 20 %.

22.5 % of the samples were naturally contaminated.

3.1.1.3 Protocols applied during the validation study

> Incubation time

- Category 1 (30 g test portion): 16 h at 37°C ± 1°C – 1/10 BPW w/o supplement.
- Category 2 (375 g test portion): 18 h at 37°C ± 1°C ¼ BPW + PIF supplement.

> Confirmations

The typical colonies were confirmed by:

- The tests described in the ISO method after subculture of typical colonies on TSA plates (1 to 5 colonies): biochemical tests (oxidase test and biochemical gallery, API ID 32E) were used during the validation study.
- PCR (iQ-Check *Cronobacter*) and biochemical gallery (API ID32E) on isolated colony without purification step.

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 ± 2 h at 41, 5°C ± 1°C.

> **Plate storage**

All the plates from positive and negative samples were stored for 72 h at 5°C ± 3°C and read again. The confirmation steps were also applied after storage (PCR and API ID32E).

> **Enrichment broth storage**

The enrichments broths were stored for 48 h at 5°C ± 3°C and streaked again onto RAPID'Sakazakii for category 1 (infant formula and infant cereals with and without probiotics including ingredients (30 g sample size)) 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 – Interpretation of sample results between the reference and alternative method (based on the confirmed alternative)

Expert lab	Category	PA	NA*	PD	ND**	PPND	PPNA	Total
QLab	1 Infant formula and infant cereals with and without probiotics including ingredients (30 g test portion)	35	23	0	2	0	0	60
ADRIA	2 Infant formula and infant cereals with and without probiotics and ingredients (375 g test portion)	25	32	9	0	0	0	66
TOTAL		60	55	9	2	0	0	126

*: PPNA not included

**: PPND not included

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

The calculations are presented in Table 5.

Table 5 – Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR)

Expert lab	Category	Type	PA	NA*	PD	ND**	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %
Qlab	1 Infant formula and infant cereals with and without probiotics including ingredients (30 g test portion)	a Infant formula and infant cereals without probiotics	11	9	0	0	0	0	100,0	100,0	100,0	0,0
		b Infant formula and infant cereals with probiotics	12	8	0	0	0	0	100,0	100,0	100,0	0,0
		c Ingredients (Maltodextrin, NFDM, whey...)	12	6	0	2	0	0	85,7	100,0	90,0	0,0
		Total	35	23	0	2	0	0	94,6	100,0	96,7	0,0
ADRIA	2 Infant formula and infant cereals with and without probiotics and ingredients (375 g test portion)	a Infant formula and infant cereals without probiotics	11	9	2	0	0	0	100,0	84,6	90,9	0,0
		b Infant formula and infant cereals with probiotics	9	11	2	0	0	0	100,0	81,8	90,9	0,0
		c Ingredients (Maltodextrin, NFDM, whey...)	5	12	5	0	0	0	100,0	50,0	77,3	0,0
		Total	25	32	9	0	0	0	100,0	73,5	86,4	0,0
Total			60	55	9	2	0	0	97,2	87,3	91,3	0,0

*: PPNA not included

**: PPND not included

A summary of the results is given in Table 6.

Table 6 - Summary of results

Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$	97.2 %
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\%$	87.3 %
Relative trueness	$RT = \frac{(PA + NA)}{N} \times 100\%$	91.3 %
False positive ratio for the alternative method* FP = PPNA + PPND	$FPR = \frac{(FP)}{NA} \times 100\%$	0.0 %

With $ND = ND + PPND$
 $NA = NA + PPNA$

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, doubtful colonies were observed on the RAPID' *Sakazakii* plates, but they were not confirmed, they were identified as *Enterobacter cloacae*.

Nine positive deviations were observed, one for naturally contaminated sample (8019: infant cereals without probiotics) and eight for artificially contaminated samples. This result could be explained by the sampling difference (375 g for the alternative method and 10 g for the reference method).

Table 7 - Negative deviations

Sample N°	Product	Reference method : ISO 22964	Alternative method : RAPID'Sakazakii				Category	Type
			RAPID'Sakazakii (direct streaking 10 µl)		Final result	Agreement		
			Typical colonies	Confirmation				
3c	Organic Brown Rice Flour	+	d (- on several plates)	-	-	ND	1	c
15c	Organic fat free milk powder	+	d (- on several plates)	-	-	ND	1	c

Table 8 - Positive deviations

Sample N°	Product	Artificial contamination		Reference method : ISO 22964*	Alternative method: RAPID'Sakazakii			Category	Type	
		Strain	Inoculation level/sample		RAPID'Sakazakii (direct streaking 10 µl)		Final result			Agreement
			Mean		Typical colonies	Confirmation				
7720	Infant cereals without probiotic multi cereals	<i>Cronobacter sakazakii</i> Ad2848	1,5	-	+p	+	+	PD	2	a
8019	Infant cereals without probiotic multi cereals	/	/	-	+p	+	+	PD	2	a
7733	Infant formula with probiotics thick formula with starch stage 2 (Bifidobacteria 8.3 x10 ⁴ CFU/g)	<i>Cronobacter turincensis</i> Ad1445	1,4	-	+p	+	+	PD	2	b
7735	Infant formula with probiotics stage 2 (L. reuteri 9.8 x10 ⁵ CFU/g)	<i>Cronobacter sakazakii</i> Ad2349	2,9	-	+p	+	+	PD	2	b
7744	Maltodextrin	<i>Cronobacter dublinensis</i> DSM18705	1,0	-	+p	+	+	PD	2	c
7745	Maltodextrin	<i>Cronobacter sakazakii</i> Ad947	0,4	-	+p	+	+	PD	2	c
7752	Lactoserum	<i>Cronobacter dublinensis</i> DSM18705	1,0	-	+p	+	+	PD	2	c
7754	Non-fat dry milk powder	<i>Cronobacter dublinensis</i> DSM18705	1,0	-	+p	+	+	PD	2	c
7755	Non-fat dry milk powder	<i>Cronobacter dublinensis</i> DSM18705	1,0	-	+p	+	+	PD	2	c

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

The analyses of discordant results according to the EN ISO 16140-2:2016 is the following (See Table 9):

Table 9 - Analyses of discordant results

Category		Type	N+	ND	PPND	PD	Unpaired study	
							(ND+PPND)-PD	AL
Qlab	1	a	11	0	0	0	0	
		b	12	0	0	0	0	
		c	14	2	0	0	2	
		Total	37	2	0	0	2	
ADRIA	2	a	13	0	0	2	-2	
		b	11	0	0	2	-2	
		c	10	0	0	5	-5	
		Total	34	0	0	9	-9	
Total			71	2	0	9	-7	5

The observed values for ((ND + PPND) - PD) meet the acceptability limit for each individual category and for the two combined categories (calculated values ≤ AL).

3.1.1.7 *Enrichment broth storage*

No change was observed after the storage at 5°C ± 3°C for 48 h for category 1 and 72h for category 2.

The analyses of discordant results remain the same as before storage (See Table 10).

Table 10 - Analysis of discordant results after enrichment storage $5 \pm 3^\circ\text{C}$

								Unpaired		
Category		Type	N+	ND	PPND	PD	(ND+PPND)-PD	AL		
Qlab	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	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	0	2	
			Total		37	2	0	0	2	3
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	0	0	2	-2	
			b	Infant formula and infant cereals with probiotics	11	0	0	2	-2	
			c	Ingredients (Maltodextrin, NFDM, whey...)	10	0	0	5	-5	
			Total		34	0	0	9	-9	3
Total			71	2	0	9	-7	5		

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

3.1.1.8 Plate broth storage at $5 \pm 3^\circ\text{C}$ for 72 h

No change was observed after the plate storage at $5^\circ\text{C} \pm 3^\circ\text{C}$ for 72 h.

3.1.1.9 Confirmation

During the validation, the typical colonies were confirmed by PCR and biochemical gallery without purification step, by biochemical tests (oxidase test and biochemical gallery) after subculture on non-selective agar plate.

When typical colonies were observed, they were identified as *Cronobacter* spp by biochemical galleries.

Doubtful colonies were observed onto RAPID' *Sakazalii* for 2 samples (3c and 15c), they were not confirmed by PCR, and they were identified as *Enterobacter cloacae*.

The typical colonies observed on the RAPID' *Sakazakii* plates from 7 samples tested after enrichment broth storage for 72 h at $5^\circ\text{C} \pm 3^\circ\text{C}$ and from 6 samples tested after plates storage for 72 h at $5^\circ\text{C} \pm 3^\circ\text{C}$ required the use of two galleries to obtain an identification (colonies tested without purification step).

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

Two (matrix/strain) pairs were analyzed by the reference method and by the alternative method (See Table 11):

The following protocol was applied:

- 0 CFU/g with 5 replicates,
- A low contamination level providing fractional recovery data, with 20 replicates,
- A higher 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 11 - Defined (matrix/strain) pairs for the RLOD determination

	Category	Matrix	Strain	Origin	Storage condition after inoculation, before analysis	Protocol	Study design
Renewal	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 + 270 mL BPW + 18 h 37°C	Unpaired
Extension	Infant formula and infant cereals with and without probiotics including ingredients (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	

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 (clause 5-1-4-2 Calculation and interpretation of RLOD) version 15.08.2015. The RLOD are given Table 12.

Table 12 – Presentation of RLOD before and after confirmation of the alternative method results

Category	(Strain / matrix) pair	Study design	AL	RLOD	RLODL	RLODU	b=ln (RLOD)	sd(b)	z-Test statistic	p-value
1	Infant cereals with probiotics / <i>Cronobacter sakazakii</i> QL 11007.9	Unpaired	2,5	0,843	0,297	2,397	-0,17	0,522	0,326	1,256
2	Infant formula with probiotics / <i>Cronobacter sakazakii</i> Ad1418			0,868	0,392	1,926	-0,141	0,398	0,354	1,277
Combined				0,870	0,468	1,617	-0,139	0,310	0,449	1,347

The LOD₅₀ % calculations according to Wilrich & Wilrich POD-LOD calculation program - version 11, 2022-10-12 test are given in Table 13.

Table 13 - LOD₅₀ results

Category	(Strain / matrix) pair	Level of detection at 50% (CFU / sample size) according to Wilrich & Wilrich ¹	
		Reference method	Alternative method
1	Infant cereals with probiotics / <i>Cronobacter sakazakii</i> QL 11007.9	1,5 [0,8; 2,9]	1,3 [0,7; 2,5]
2	Infant formula with probiotics / <i>Cronobacter sakazakii</i> Ad1418	1,0 [0,6; 1,8]	0,9 [0,5; 1,6]
Combined results		1,3 [0,9; 1,8]	1,2 [0,9; 1,7]

3.1.3 Conclusion

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

The LOD₅₀ varies from 1.0 to 1.5 CFU/sample size for the reference method and from 0.9 to 1.3 CFU/ sample size for the alternative method.

¹ Wilrich, C., and P.-Th. Wilrich: Estimation of the POD function and the LOD of a qualitative microbiological measurement method. AOAC International **92** (2009) 1763 - 1772.

3.1.4 Inklusivity / 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.

3.1.4.1 Test protocols

> Inklusivity

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 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.

3.1.4.2 Results

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

> Inklusivity

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

> Exclusivity

The 31 non-target strains showed negative results.

3.1.5 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	They are provided on the package and on the plates. The plates are stored at 2 – 8°C.		
Time to result	Steps	Reference method	Alternative method
	Negative samples		
	Pre-enrichment	Day 0	Day 0
	Enrichment 1	Day 1	/
	Streaking	Day 2	Day 1
	Reading	Day 3	Day 2
	Presumptive positive or positive results		
	Pre-enrichment	Day 0	Day 0
	Enrichment 1	Day 1	/
	Streaking	Day 2	Day 1
	Reading	Day 3	Day 2
	Streaking onto TSA plates	Day 4	Day 3
	Confirmatory test	Day 6	Day 5
	The results are obtained one day earlier by the alternative method than by the reference method, due to the absence of subculture in CSB broth.		
	Common step with the reference method	Pre-enrichment is common to both methods.	

The negative results are available in 2 days and the positive results in 5 days using the RAPID[®] *sakazakii* 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

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

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

- 24 labelled samples (30 ml) for *Cronobacter spp.* detection by RAPID' *Sakazakii* method and by the ISO/TS 22964 (2006) reference method for the detection of *Enterobacter sakazakii*,
- 1 unlabeled 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.

Blind 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*

Strain stability was checked by inoculating the matrix at 10 CFU/30ml and 1 CFU/30 ml and 100CFU/30ml. Enumerations were performed for the high contamination level and detection analyses were performed for the low contamination level after 24 h and 48 h storage at $5 \pm 3^\circ\text{C}$. *Triplicates* were analysed. The aerobic mesophilic flora was also enumerated; the results are given in 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 $5^\circ\text{C} \pm 3^\circ\text{C}$.

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 (CFU/30 ml)	True level (CFU/30 ml sample)	Low limit CFU / 30 ml sample	High limit CFU / 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 ²	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.

² Temperature measured after the sample storage at 4°C

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.

Table 17 – Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	0/8	0/8
L1	4/8	4/8
L2	8/8	8/8

All the control samples gave a negative result and all the samples inoculated at high level gave positive results by the reference and the alternative methods. For the low inoculation level, fractional positive results were observed: 4 samples gave positive results by the reference and the alternative methods.

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 $2,0 \cdot 10^5$ CFU/ml.

> ***Cronobacter spp. detection***

The Lab J started the analyses on 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 interpretation. Cross contaminations were observed in the blank samples for many Labs: 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 results obtained by the 13 collaborators are provided in Table 18 (reference method) and Table 19 (alternative method).

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

Collaborator	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	0	0	0	1	1	1	8	8	8
B	0	0	0	2	2	2	8	8	8
C	1	1	1	5	5	5	8	8	8
D	0	0	0	4	4	4	8	8	8
E	0	0	0	6	6	6	8	8	8
F	0	0	0	5	5	5	8	8	8
G	2	2	2	3	3	3	8	8	8
H	3	3	3	7	7	7	8	8	8
I	0	0	0	5	5	5	8	8	8
J	0	0	0	6	6	6	8	8	8
K	0	0	0	3	3	3	8	8	8
L	0	0	0	3	3	3	8	8	8
M	1	1	1	3	3	3	8	8	8
Total	P₀=7	C₀=7	CP₀=7	P₁=53	C₁=53	CP₁=53	P₂=104	C₂=104	CP₂=104

According to the AFNOR technical rules, it is possible to include the results from a collaborator with maximum one presumptive positive or confirmed positive sample at Level 0. For this study, the rule was applied, and Labs C, G and H were excluded.

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

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
I	0	5	8
J	0	6	8
K	0	3	8
L	0	3	8
M	0	3	8
TOTAL	P₀ = 1	P₁ = 39	P₂ = 80

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

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	0	0	0	1	1	1	8	8	8
B	0	0	0	2	2	2	8	8	8
D	0	0	0	4	4	4	8	8	8
E	0	0	0	6	6	6	8	8	8
F	0	0	0	5	5	5	8	8	8
I	0	0	0	5	5	5	8	8	8
J	0	0	0	6	6	6	8	8	8
K	0	0	0	3	3	3	8	8	8
L	0	0	0	3	3	3	8	8	8
M	1	1	1	3	3	3	8	8	8
Total	P₀ = 1	C₀=1	CP₀=1	P₁ = 38	C₁=38	CP₁=38	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	$SP_{ref} = \left(1 - \left(\frac{P_0}{N_-}\right)\right) \times 100 \% =$	98.8%
Specificity for the alternative method	$SP_{alt} = \left(1 - \left(\frac{CP_0}{N_-}\right)\right) \times 100 \% =$	98.8 %

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) and the false positive ratio for the alternative method (FPR)

Fractional positive results were obtained for the low inoculation level (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 the obtained results with the reference method and the alternative method for Level 1 and Level 2

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	Alternative method positive (A+)	Positive agreement (A+/R+) PA = 38	Positive deviation (R-/A+) PD = 0
	Alternative method negative (A-)	Negative deviation (A-/R+) ND = 1 (PPND = 0)	Negative agreement (A-/R-) NA = 41 (PPNA = 0)

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

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

		Level 1
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA+PD)}{(PA+PD+ND)} \times 100\% =$	97.4%
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA+ND)}{(PA+PD+ND)} \times 100\% =$	100%
Relative trueness	$RT = \frac{(PA+NA)}{N} \times 100\% =$	98.8%
False positive ratio for the alternative method	$FPR = \frac{FP}{NA} \times 100\% =$	0%

3.2.4.3 Interpretation of data

The negative deviations are listed in Table 25 for Level 1. No positive deviation was observed for Level 1.

Table 25 - Negative deviations for Level 1

Lab	Level	Sample n°	RAPID'Sakazakii	
			Typical colonies	Confirmation
F	1	F18	-	-

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

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

where

P_x = number of samples with a positive result obtained with the reference method at level x (L_1 or L_2) for all the collaborators

N_x = number of samples tested at level x (L_1 or L_2) with the reference method by all the collaborators

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

where

CP_x = number of samples with a confirmed positive result obtained with the alternative method at level x (L_1 or L_2) for all the collaborators;

N_x = number of samples tested at level x (L_1 or L_2) with the alternative method by all the collaborators.

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

where

N_x = number of samples tested for level x (L_1 or L_2) with the reference method by all the collaborators.

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 EN ISO 16140-2:2016 (See Table 26).

Table 26 - Calculations

	Level 1
N_x	80
$(\rho^+)_{ref}$	0.5
$(\rho^+)_{alt}$	0.5
AL = (ND - PD) max	10.95
ND - PD	1
Conclusion	ND-PD<AL

The ISO 16140-2 (2016) requirements are fulfilled as ND - PD meet the AL.

There is indeed no difference between the sensitivity of the compared methods, and the alternative method complies with the reproducibility conditions.

3.2.4.4 Evaluation of the $LOD_{50\%}$, $LOD_{95\%}$ and RLOD between laboratories

The $LOD_{50\%}$, the $LOD_{95\%}$ and the RLOD was calculated using the EN ISO 16140-2:2016 Excel spreadsheet available at https://standards.iso.org/iso/16140/-5/ed-1/en/RLOD_inter-lab-study_16140-2_AnnexF_ver1_28-06-2017.xls. The results are used only for information (see Table 27).

Table 27 - $LOD_{50\%}$, $LOD_{95\%}$ and RLOD

Method	$LOD_{50\%}$	$LOD_{95\%}$	RLOD
Reference	1.1 [0.8;1.6]	4.7 [3.3;6.9]	1 [0.7;1.5]
Alternative	1.1 [0.8;1.6]	4.7 [3.3;6.9]	

3.3 General conclusion

The **method comparison study conclusions** are:

- The method comparison study scheme corresponds to an UNPAIRED STUDY design as the alternative and reference methods have different enrichment procedures.
- In the sensitivity study, two categories were tested. The protocol of the alternative method shows 9 positive deviations (PD) and 2 negative deviations (ND) for the two categories tested. The ND - PD meet the acceptability limits (AL) for each category, and as well for the two tested categories.
- The Relative Levels of Detection (RLOD) are all below the AL fixed at 2.5 for the unpaired data study whatever the matrix/strain pairs.
- The inclusivity and exclusivity testing gave the expected results for the 52 target strains and the 31 non-target strains.
- 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 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 EN ISO 16140-2:2016 requirements. **The RAPID' *Sakazakii* method is considered equivalent to the ISO standard.**

Quimper, 11 June 2024

Astrid CARIOU
Manager

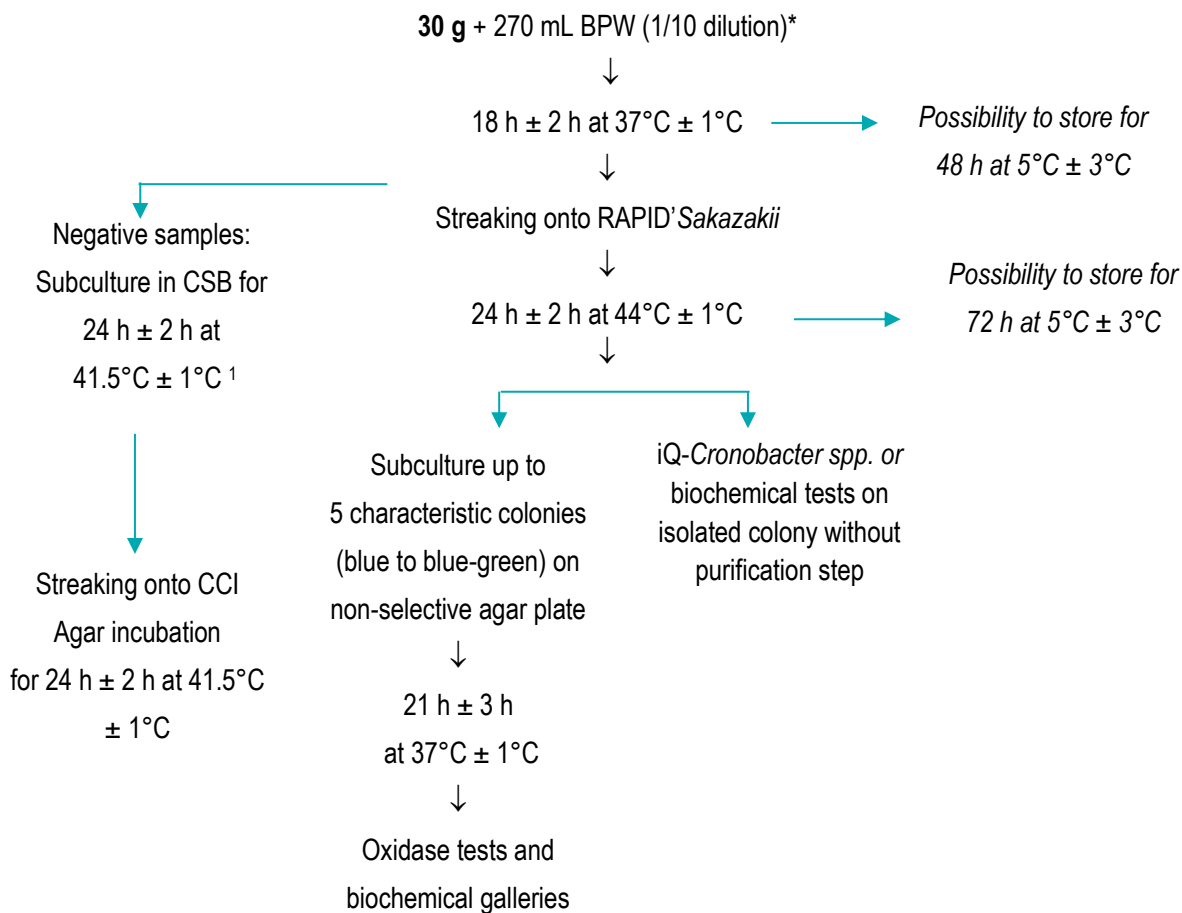
Validation of Alternative methods

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: RAPID' *Sakazaki*

**Infant formula and infant cereals with
and without probiotics including ingredients (30 g sample size)**

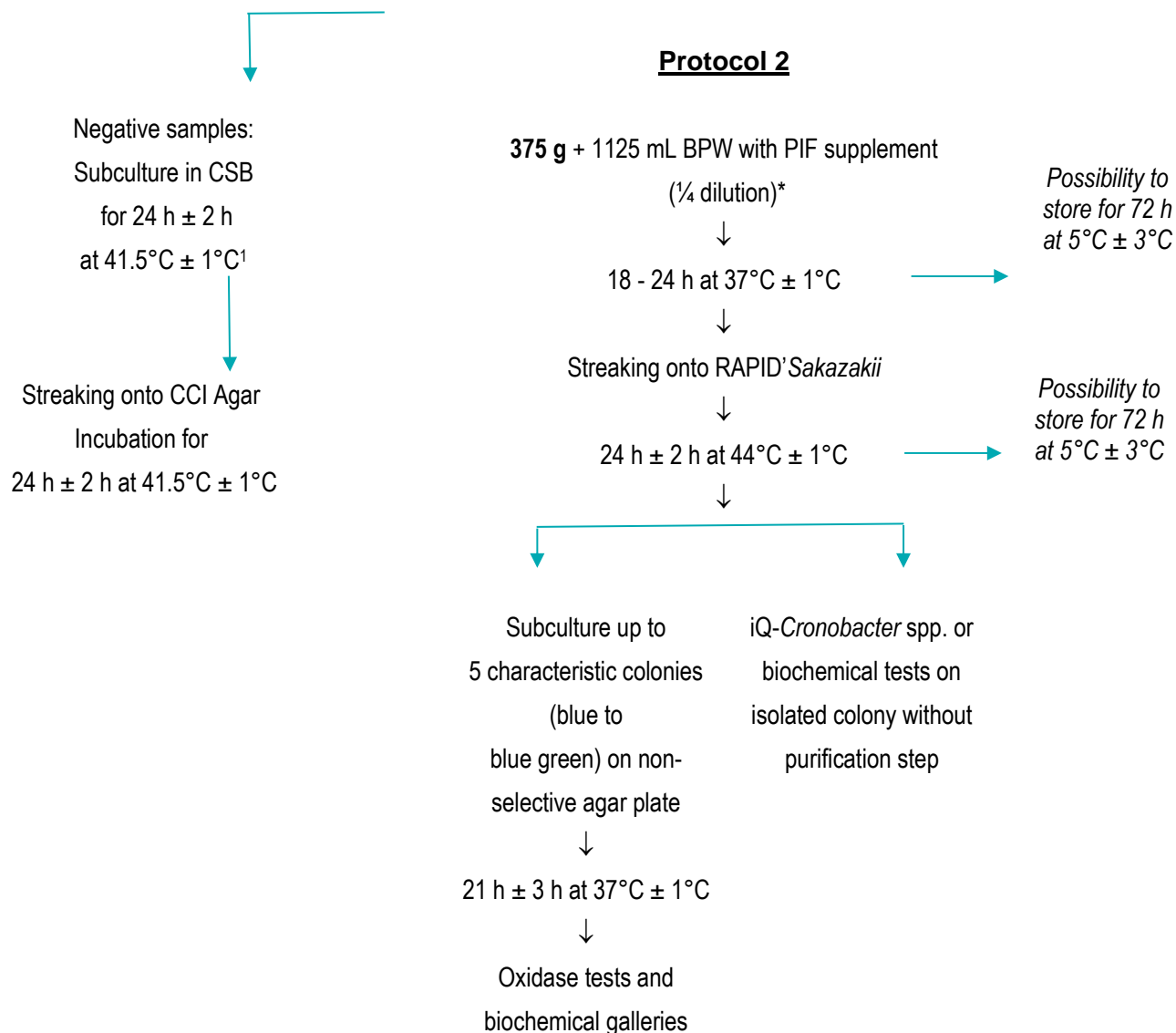
Protocol 1



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

¹ This protocol was applied during the validation study in the sensitivity part for negative samples as requested by the ISO 16140-2 (2016)

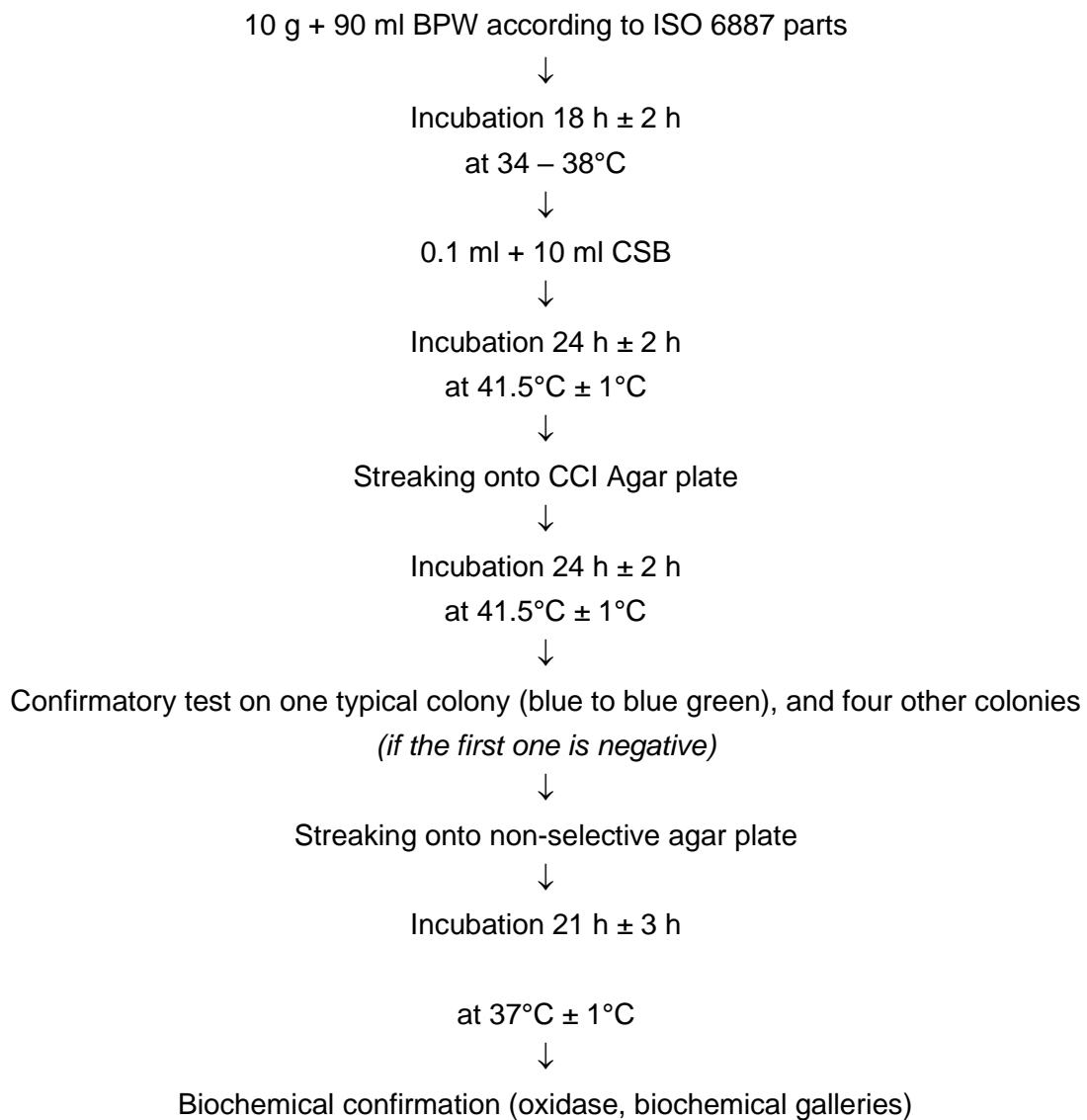
**Infant formula and infant cereals with and without probiotics and ingredients
(375 g sample size)**



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

¹ This protocol was applied during the validation study in the sensitivity part for negative samples as requested by the ISO 16140-2 (2016)

**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.**



Appendix 3 – Artificial contamination of samples

Year of analysis	Sample N°	Product	Artificial contamination (Q-Laboratories Inc.)						Global result	Category	Type
			Strain	Origine	Injury protocol	Injury measurement	Inoculation level/sample				
							Enumeration	Mean			
2020	6a	Multigrain banana infant cereal	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	2	2	+	1	a
2020	9a	Oatmeal banana infant cereal	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	4-5-3-5-4	4,2	+	1	a
2020	10a	single grain raisin infant cereal	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	4-5-3-5-4	4,2	+	1	a
2020	12a	Pure Bliss Infant Formula	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	4-5-3-5-4	4,2	+	1	a
2020	14a	Infant formula with iron, 0-12 Months	<i>Cronobacter condimenti</i> QL 17031.1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	4-5-3-5-4	4,2	+	1	a
2020	20a	Organic Stage 2 infant formula	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	3-3-5-2-6	3,8	+	1	a
2020	2b	Rice and banana apple - infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	3-3-5-2-6	3,8	+	1	b
2020	3b	Multi-Grain - infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	3-3-5-2-6	3,8	+	1	b
2020	6b	Oats and Quinoa - infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	3-3-5-2-6	3,8	+	1	b
2020	8b	Oatmeal Infant cereal with probiotics	<i>Cronobacter sakazakii</i> QL 120117-1	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	3-3-5-2-6	3,8	+	1	b
2020	13b	Good Start - infant formula with probiotics	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	5-3-7-4-5	4,8	+	1	b
2020	18b	Sensitive - infant formula with probiotics	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	5-3-7-4-5	4,8	+	1	b
2020	19b	Fat malabsorption problems - infant formula with probiotics	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	5-3-7-4-5	4,8	+	1	b
2020	1c	Organic Coconut oil	<i>Cronobacter sakazakii</i> FSL F6-044	Food	Heat treatment (50 - 55°C for 10-15 minutes)	0,56	4-2-3	3	+	1	c
2020	2c	Whole Wheat Flour	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	5-3-7-4-5	4,8	+	1	c
2020	5c	Old Fashioned Oats	<i>Cronobacter turicensis</i> QL 17031.5	Infant Formula	Storage for 2 weeks at room temperature (20-25°C)	/	5-3-7-4-5	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	4-2-3	3	+	1	c
2020	11c	Non-fat dry milk powder	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Storage for 2 weeks at room temperature (20-25°C)	/	3-2-4-1	2,5	+	1	c
2020	13c	Instant milk powder	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Storage for 2 weeks at room temperature (20-25°C)	/	3-2-4-1	2,5	+	1	c
2020	14c	Skim milk powder	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Storage for 2 weeks at room temperature (20-25°C)	/	3-2-4-1	2,5	+	1	c
2020	16c	Whey protein	<i>Cronobacter sakazakii</i> QL 111717-2	Powdered Milk	Storage for 2 weeks at room temperature (20-25°C)	/	3-2-4-1	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	4-2-3	3	+	1	c

Year of analysis	Sample N°	Product	Product (French name)	Artificial contaminations (ADRIA)					Global result	Category	Type
				Strain	Origin	Injury protocol	Injury measurement	Inoculation level			
								CFU/sample			
					Mean						
2019	7720	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,5	+	2	a
2019	7721	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	<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	Céréales infantiles sans probiotique 5 céréales	<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+)	Céréales infantiles sans probiotique babivanille (10+ mois)	<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	Céréales infantiles sans probiotique chocobiscuité	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,5	+	2	a
2019	7725	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	<i>Cronobacter sakazakii</i> Ad2848	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,5	+	2	a
2019	7726	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2,3	+	2	a
2019	7727	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2,3	+	2	a
2019	7728	Infant formula stage 2 organic	Poudre de lait infantile bio 2ème âge sans probiotique	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2,3	+	2	a
2019	7729	Follow-up infant formula	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2,3	+	2	a
2019	7730	Infant formula stage 1	Poudre de lait infantile sans probiotique premier âge	<i>Cronobacter sakazakii</i> Ad1418	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	2,3	+	2	a
2019	7731	Infant formula stage 2	Poudre de lait infantile sans probiotique 2ème âge	<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 (B.infantis 8,3.10 ⁴ CFU/g)	Poudre de lait avec probiotiques 2ème âge (B.infantis 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 (Bifidobacteria 8,3.10 ⁴ CFU/g)	Poudre de lait avec probiotiques formule épaissie amidon 2ème âge (Bifidobactéries 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 (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)	<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 (L. reuteri 9,8.10 ⁵ CFU/g)	Poudre de lait avec probiotiques relais 2ème âge (L. reuteri 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 (Bifidobacteria 1,5.10 ³ CFU/g)	Poudre de lait avec probiotiques formule épaissie 2ème âge (Bifidobactéries 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 (B.lactis 5,2.10 ⁵ CFU/g)	Poudre de lait avec probiotiques 2ème âge (B.lactis 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 (B. lactis 5,3.10 ⁵ CFU/g)	Céréales infantiles vanille avec probiotiques (B.lactis 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 (B. lactis 2,7.10 ⁶ CFU/g)	Céréales infantiles 5 céréales avec probiotiques (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	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)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,1	+	2	b
2019	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)	<i>Cronobacter sakazakii</i> Ad2849	Cereals	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,1	+	2	b
2019	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)	<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)	Céréales infantiles noisette biscuit avec probiotiques (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

Year of analysis	Sample N°	Product	Product (French name)	Artificial contaminations (ADRIA)					Global result	Category	Type
				Strain	Origin	Injury protocol	Injury measurement	Inoculation level			
								CFU/sample			
					Mean						
2019	7744	Maltodextrin	Maltodextrine	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,0	+	2	c
2019	7745	Maltodextrin	Maltodextrine	<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	Poudre de lait écrémé	<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	Poudre de lait écrémé	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0,4	+	2	c
2019	7748	Whey	Lactoserum	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,0	-	2	c
2019	7749	Whey	Lactoserum	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0,4	-	2	c
2019	7750	Whey	Lactoserum	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0,4	+	2	c
2019	7751	Maltodextrin	Maltodextrine	<i>Cronobacter sakazakii</i> Ad947	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	0,4	+	2	c
2019	7752	Lactoserum	Lactoserum	<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	Poudre de lait écrémé	<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	Poudre de lait écrémé	<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	Poudre de lait écrémé	<i>Cronobacter dublinensis</i> DSM18705	Milk powder	Seeding Lyophilised strain 2 weeks ambient temperature	/	1,0	+	2	c

Appendix 4 – Sensitivity study: raw data**Cronobacter detection results:**

+m:	minority level of target analyte
+M :	majority level of target analyte
+p:	pure culture level of target analyte
+1/2 :	50% level of target analyte
-:	no typical colonies but presence of background microflora
st:	plate without any colony
d:	doubtful colony
ni:	non-isolated colonies
NC:	non-characteristic colony on nutrient agar
i:	PCR inhibition
PA:	positive agreement
NA:	negative agreement
ND:	negative deviation
PD:	positive deviation
PPNA:	positive presumptive negative agreement
PPND:	positive presumptive negative deviation
NI:	no identification
*:	1:10 dilution of the DNA extract
I.C.:	Internal control
FDRS:	Free DNA Removal Solution

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

Year of analysis	Sample N°	Product	Products (French name)	Reference method				Alternative method: RAPID'Sakazakii													Category	Type
								30 g + 270 mL BPW - 16 h 2 h at 37°C ± 1°C														
								RAPID'Sakazakii (direct streaking 10 µl)														
								Confirmation														
								Without purification step						With purification step								
CCI	Oxidase	Identification	Result	Typical colonies	Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/ APF Fast)			Oxidase	Biochemical gallery (API ID32)	Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final result	Agreement							
						Cq	I.C. Cq	Final result														
2020	1a	Organic baby cereal	Céréales infantiles bio	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,24	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	2a	Step 1 infant cereal	Céréales infantiles 1er âge	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	3a	Step 3 infant cereal	Céréales infantiles 3ème âge	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	4a	Stage 1 infant cereal	Céréales infantiles 1er âge	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	5a	Stage 2 infant cereal	Céréales infantiles 2ème âge	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,58	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	6a	Multigrain banana infant cereal	Céréales infantiles multigraines, banane	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	30,92	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	7a	Oatmeal	Avoine infantile	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	8a	Single grain infant cereal	Céréales infantiles	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	9a	Oatmeal banana infant cereal	Avoine infantiles, banane	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,38	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	10a	single grain raisin infant cereal	Céréales infantiles raisins	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,21	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	11a	Neosure infant formula	Poudre de lait infantiles	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	12a	Pure Bliss Infant Formula	Poudres de lait infantiles	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,42	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	13a	State 1 Non-GMO Infant Formula	Poudres de lait infantiles, 1er âge	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,55	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	14a	Infant formula with iron, 0-12 Months	Poudre de lait infantiles, 0-12 mois, avec fer	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,47	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	15a	Stage 23 infant formula	Poudre de lait infantiles, 1er-2ème âge	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	16a	Soy based infant formula	Poudre de lait infantiles, soja	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	17a	Non-GMO Infant Formula	Poudre de lait infantiles sans OGM	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,69	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	18a	Infant formula with iron	Poudre de lait infantiles, avec fer	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,42	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	19a	Organic infant formula with lactose	Poudre de lait infantiles, avec lactose	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	a	
2020	20a	Organic Stage 2 infant formula	Poudre de lait infantiles, 2ème âge	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,24	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	a	
2020	1b	Frawler - infant cereal with probiotics (8,2.10 ⁵ CFU/g)	Céréales infantiles avec probiotiques	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	2b	Rice and banana apple - infant cereal with probiotics (4,9.10⁶ CFU/g)	Céréales infantiles avec probiotiques, riz, banane, pomme	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,4	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	b	
2020	3b	Multi-Grain - infant cereal with probiotics (6,5.10⁶ CFU/g)	Céréales infantiles avec probiotiques, multi-graines	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,59	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	b	

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (30 g sample size) - Study realized by Q-Laboratories Inc.																						
Year of analysis	Sample N°	Product	Products (French name)	Reference method				Alternative method: RAPID'Sakazakii													Category	Type
				30 g + 270 mL BPW - 16 h 2 h at 37°C ± 1°C																		
				RAPID'Sakazakii (direct streaking 10 µl)																		
				Confirmation																		
CCI	Oxidase	Identification	Result	Typical colonies	Without purification step					With purification step			Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final result	Agreement						
					Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/ APF Fast)			Oxidase	Biochemical gallery (API ID32)											
						Cq	I.C. Cq	Final result														
2020	4b	Organic with DHA - Infant Cereal with probiotics (2,3.10 ⁶ CFU/g)	Céréales infantiles avec probiotiques, bio avec DHA	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,26	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	5b	Single grain rice - infant cereal with probiotics (5,0.10 ⁶ CFU/g)	Céréales infantiles avec probiotiques, riz	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,3	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	6b	Oats and Quinoa - infant cereal with probiotics (8,9.10 ⁵ CFU/g)	Céréales infantiles avec probiotiques, avoine et quinoa	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	25	31,41	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	7b	Singlegrain variety pack - infant cereal with probiotics (8,2.10 ⁶ CFU/g)	Céréales infantiles avec probiotiques	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	8b	Oatmeal Infant cereal with probiotics (1,3.10 ⁶ CFU/g)	Avoine infantile avec probiotiques	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,34	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	9b	Organic oatmeal with choline - infant cereal with probiotics (1,8.10 ⁶ CFU/g)	Céréales infantiles avec probiotiques, avoine	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	10b	Oatmeal banana strawberry - infant cereal with probiotics (4,5.10 ⁶ CFU/g)	Céréales infantiles avec probiotiques, avoine, banane, fraise	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,53	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	11b	Comfort - infant formula with probiotics (9,6.10 ⁵ CFU/g)	Poudre de lait infantiles avec probiotiques	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	12b	With iron - infant formula with probiotics (7,2.10 ⁵ CFU/g)	Poudre de lait infantiles avec probiotiques avec fer	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	13b	Good Start - infant formula with probiotics (6,8.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques avec fer	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	34,68	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	14b	For spit up - infant formula with probiotics (8,8.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques avec fer	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	15b	Total comfort - infant formula with probiotics (2,9.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques avec fer	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b	
2020	16b	Organic Lactose - infant formula with probiotics (5,7.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques, avec lactose bio	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,53	+	-	Cronobacter spp.	/	+	+	PA	1	b	
2020	17b	Milk based - infant formula with probiotics (3,6.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,14	+	-	Cronobacter spp.	/	+	+	PA	1	b	

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

Year of analysis	Sample N°	Product	Products (French name)	Reference method				Alternative method: RAPID'Sakazakii												Category	Type
								30 g + 270 mL BPW - 16 h 2 h at 37°C ± 1°C													
								RAPID'Sakazakii (direct streaking 10 µl)													
								Confirmation						Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final result	Agreement				
CCI	Oxidase	Identification	Result	Typical colonies	Without purification step			With purification step													
					Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/ APF Fast)		Oxidase	Biochemical gallery (API ID32)											
						Cq	I.C. Cq	Final result													
2020	18b	Sensitive - infant formula with probiotics (7,8.10 ⁵ CFU/g)	Poudre de lait infantiles avec probiotiques	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,43	+	-	Cronobacter spp.	/	+	+	PA	1	b
2020	19b	Fat malabsorption problems - infant formula with probiotics (3,1.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques, problème d'absorption de la matière grasse	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,25	+	-	Cronobacter spp.	/	+	+	PA	1	b
2020	20b	Gentle start - infant formula with probiotics (6,4.10 ⁶ CFU/g)	Poudre de lait infantiles avec probiotiques, 1er âge	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	b
2020	1c	Organic Coconut oil	Huile de coco bio	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,58	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	2c	Whole Wheat Flour	Farine de blé entier	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	27	31,6	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	3c	Organic Brown Rice Flour	Farine de riz bio	+1/2	-	Cronobacter spp.	+	d (- on several plates)	-	Enterobacter cloacae	-	32,27	-	-	Enterobacter cloacae	-	-	-	ND	1	c
2020	4c	Quinoa Flour	Farine de quinoa	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	c
2020	5c	Old Fashioned Oats	Avoine	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,51	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	6c	Brown Rice Flour	Farine de riz	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	29	31,62	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	7c	All purpose Flour	Farine	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	30	32,02	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	8c	Organic Soy Oil	Huile de soja bio	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	30	32,18	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	9c	Sodium Citrate	Citrate de sodium	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	c
2020	10c	Folic Acid	Acide folique	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	c
2020	11c	Non-fat dry milk powder	Poudre de lait écrémé	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	30	31,96	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	12c	Organic goat milk powder	Poudre de lait de chèvre bio	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	c
2020	13c	Instant milk powder	Poudre de lait	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	29	31,47	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	14c	Skim milk powder	Poudre de lait écrémé	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	25	31,09	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	15c	Organic fat free milk powder	Poudre de lait écrémé bio	+1/2	-	Cronobacter spp.	+	d (- on several plates)	-	Enterobacter cloacae	-	32,52	-	-	Enterobacter cloacae	-	-	-	ND	1	c
2020	16c	Whey protein	Lactosérum	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	29	32,16	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	17c	Organic sunflower oil	Huile de tournesol bio	+1/2	-	Cronobacter spp.	+	+M	-	Cronobacter spp.	28	31,63	+	-	Cronobacter spp.	/	+	+	PA	1	c
2020	18c	Manganese sulfate	Sulfate de manganese	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	c

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								30 g + 270 mL BPW - 16 h 2 h at 37°C ± 1°C													
								RAPID'Sakazakii (direct streaking 10 µl)													
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				CCI	Oxidase	Identification	Result	Typical colonies	Without purification step					With purification step		Negative samples (Subculture CSB+ streaking onto CCI)	All confirmatory tests	Final result	Agreement		
									Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/ APF Fast)			Oxidase	Biochemical gallery (API ID32)						
								Cq			I.C. Cq	Final result									
2020	19c	Sinc sulfate	Sulfate de zinc	+1/2	-	<i>Cronobacter</i> spp.	+	+M	-	<i>Cronobacter</i> spp.	28	31,59	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	1	c
2020	20c	Biotin	Biotine	-	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	1	c

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

Year of analysis	Sample N°	Product	Reference method: Result	Alternative method: RAPID'Sakazakii																Category	Type					
				30 g + 270 mL BPW 16 h at 37°C ± 1°C + enrichment broth storage for 48h at 5 ±3 °C									30 g + 270 mL BPW 16 h at 37°C ± 1°C + plates storage for 72h at 5 ±3 °C													
				RAPID'Sakazakii (direct streaking 10 µl)									Rapid'sakazakii (direct streaking 10 µl)													
				Typical colonies	Confirmation								All confir-matory tests	Final result	Agree-ment	Typical colonies	Confirmation					All confir-matory tests	Final result	Agree-ment		
					Without purification step												Without purification step									
Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)			Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)																			
		Cq	I.C. Cq	Final result			Cq	I.C. Cq	Final result																	
2020	1a	Organic baby cereal	+	+M	-	<i>Cronobacter</i> spp.	26	32,04	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,86	+	+	+	PA	1	a			
2020	2a	Step 1 infant cereal	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	3a	Step 3 infant cereal	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	4a	Stage 1 infant cereal	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	5a	Stage 2 infant cereal	+	+M	-	<i>Cronobacter</i> spp.	26	31,72	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,48	+	+	+	PA	1	a			
2020	6a	Multigrain banana infant cereal	+	+M	-	<i>Cronobacter</i> spp.	28	30,65	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	25	30,52	+	+	+	PA	1	a			
2020	7a	Oatmeal	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	8a	Single grain infant cereal	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	9a	Oatmeal banana infant cereal	+	+M	-	<i>Cronobacter</i> spp.	26	32,11	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,96	+	+	+	PA	1	a			
2020	10a	single grain raisin infant cereal	+	+M	-	<i>Cronobacter</i> spp.	26	32,47	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,57	+	+	+	PA	1	a			
2020	11a	Neosure infant formula	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	12a	Pure Bliss Infant Formula	+	+M	-	<i>Cronobacter</i> spp.	26	31,98	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,53	+	+	+	PA	1	a			
2020	13a	State 1 Non-GMO Infant Formula	+	+M	-	<i>Cronobacter</i> spp.	26	31,62	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	28	31,52	+	+	+	PA	1	a			
2020	14a	Infant formula with iron, 0-12 Months	+	+M	-	<i>Cronobacter</i> spp.	26	32,2	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,75	+	+	+	PA	1	a			
2020	15a	Stage 23 infant formula	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	16a	Soy based infant formula	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	17a	Non-GMO Infant Formula	+	+M	-	<i>Cronobacter</i> spp.	26	31,97	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,52	+	+	+	PA	1	a			
2020	18a	Infant formula with iron	+	+M	-	<i>Cronobacter</i> spp.	26	31,89	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,4	+	+	+	PA	1	a			
2020	19a	Organic infant formula with lactose	-										-	/	/	/	/	/	/	-	NA	1	a			
2020	20a	Organic Stage 2 infant formula	+	+M	-	<i>Cronobacter</i> spp.	25	31,63	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	28	31,52	+	+	+	PA	1	a			
2020	1b	Frawler - infant cereal with probiotics (8,2.10 ⁵ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b			
2020	2b	Rice and banana apple - infant cereal with probiotics (4,9.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	25	30,85	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	29,16	+	+	+	PA	1	b			
2020	3b	Multi-Grain - infant cereal with probiotics (6,5.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	25	30,88	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	32,08	+	+	+	PA	1	b			
2020	4b	Organic with DHA - Infant Cereal with probiotics (2,3.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	24	28,48	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,9	+	+	+	PA	1	b			
2020	5b	Single grain rice - infant cereal with probiotics (5,0.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	25	30,52	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,55	+	+	+	PA	1	b			
2020	6b	Oats and Quinoa - infant cereal with probiotics (8,9.10 ⁵ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	26	31,87	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,49	+	+	+	PA	1	b			

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

Year of analysis	Sample N°	Product	Reference method: Result	Alternative method: RAPID'Sakazakii															Category	Type			
				30 g + 270 mL BPW 16 h at 37°C ± 1°C + enrichment broth storage for 48h at 5 ±3 °C									30 g + 270 mL BPW 16 h at 37°C ± 1°C + plates storage for 72h at 5 ±3 °C										
				RAPID'Sakazakii (direct streaking 10 µl)									Rapid'sakazakii (direct streaking 10 µl)								All confir- matory tests	Final result	Agree- ment
				Typical colonies	Confirmation								Typical colonies	Confirmation									
					Without purification step									Without purification step									
Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)			All confir- matory tests	Final result	Agree- ment	Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)													
		Cq	I.C. Cq	Final result						Cq	I.C. Cq	Final result											
2020	7b	Singlegrain variety pack - infant cereal with probiotics (8,2.10 ⁶ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	8b	Oatmeal Infant cereal with probiotics (1,3.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	26	31,71	+	+	+	PA	+M	-	<i>Cronobacter</i> spp..	29	31,6	+	+	+	PA	1	b
2020	9b	Organic oatmeal with choline - infant cereal with probiotics (1,8.10 ⁶ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	10b	Oatmeal banana strawberry - infant cereal with probiotics (4,5.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	26	31,85	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	32,01	+	+	+	PA	1	b
2020	11b	Comfort - infant formula with probiotics (9,6.10 ⁵ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	12b	With iron - infant formula with probiotics (7,2.10 ⁵ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	13b	Good Start - infant formula with probiotics (6,8.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	26	32,12	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,81	+	+	+	PA	1	b
2020	14b	For spit up - infant formula with probiotics (8,8.10 ⁶ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	15b	Total comfort - infant formula with probiotics (2,9.10 ⁶ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	16b	Organic Lactose - infant formula with probiotics (5,7.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	26	32,51	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	32,01	+	+	+	PA	1	b
2020	17b	Milk based - infant formula with probiotics (3,6.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	30	31,72	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,59	+	+	+	PA	1	b
2020	18b	Sensitive - infant formula with probiotics (7,8.10 ⁵ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	26	32,1	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,17	+	+	+	PA	1	b
2020	19b	Fat malabsorption problems - infant formula with probiotics (3,1.10 ⁶ CFU/g)	+	+M	-	<i>Cronobacter</i> spp.	25	31,94	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	29	31,33	+	+	+	PA	1	b
2020	20b	Gentle start - infant formula with probiotics (6,4.10 ⁶ CFU/g)	-										-	/	/	/	/	/	/	-	NA	1	b
2020	1c	Organic Coconut oil	+	+M	-	<i>Cronobacter</i> spp.	29	31,83	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,22	+	+	+	PA	1	c
2020	2c	Whole Wheat Flour	+	+M	-	<i>Cronobacter</i> spp.	29	31,55	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,55	+	+	+	PA	1	c
2020	3c	Organic Brown Rice Flour	+	d (- on several plates)	-	<i>Enterobacter cloacae</i>	-	32,26	-	-	-	ND	d	-	<i>Enterobacter cloacae</i>	-	32	-	-	-	ND	1	c
2020	4c	Quinoa Flour	-										-	/	/	/	/	/	/	-	NA	1	c

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

Year of analysis	Sample N°	Product	Reference method: Result	Alternative method: RAPID'Sakazakii																Category	Type					
				30 g + 270 mL BPW 16 h at 37°C ± 1°C + enrichment broth storage for 48h at 5 ±3 °C									30 g + 270 mL BPW 16 h at 37°C ± 1°C + plates storage for 72h at 5 ±3 °C													
				RAPID'Sakazakii (direct streaking 10 µl)									Rapid'sakazakii (direct streaking 10 µl)													
				Typical colonies	Confirmation								All confir-matory tests	Final result	Agree-ment	Typical colonies	Confirmation					All confir-matory tests	Final result	Agree-ment		
					Without purification step												Without purification step									
Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)			Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)																			
		Cq	I.C. Cq	Final result			Cq	I.C. Cq	Final result																	
2020	5c	Old Fashioned Oats	+	+M	-	<i>Cronobacter</i> spp.	29	31,27	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,14	+	+	+	PA	1	c			
2020	6c	Brown Rice Flour	+	+M	-	<i>Cronobacter</i> spp.	29	31,59	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,22	+	+	+	PA	1	c			
2020	7c	All purpose Flour	+	+M	-	<i>Cronobacter</i> spp.	29	31,51	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,23	+	+	+	PA	1	c			
2020	8c	Organic Soy Oil	+	+M	-	<i>Cronobacter</i> spp.	29	31,33	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,17	+	+	+	PA	1	c			
2020	9c	Sodium Citrate	-										-	/	/	/	/	/	/	-	NA	1	c			
2020	10c	Folic Acid	-										-	/	/	/	/	/	/	-	NA	1	c			
2020	11c	Nonfat dry milk powder	+	+M	-	<i>Cronobacter</i> spp.	28	31,11	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,47	+	+	+	PA	1	c			
2020	12c	Organic goat milk powder	-			<i>Cronobacter</i> spp.							-	/	/	/	/	/	/	-	NA	1	c			
2020	13c	Instant milk powder	+	+M	-	<i>Cronobacter</i> spp.	29	31,43	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	30,95	+	+	+	PA	1	c			
2020	14c	Skim milk powder	+	+M	-	<i>Cronobacter</i> spp.	29	31,32	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,45	+	+	+	PA	1	c			
2020	15c	Organic fat free milk powder	+	d (- on several plates)	-	<i>Enterobacter cloacae</i>	-	32,33	-	-	-	ND	d	-	<i>Enterobacter cloacae</i>	-	31,61	-	-	-	ND	1	c			
2020	16c	Whey protein	+	+M	-	<i>Cronobacter</i> spp.	29	31,33	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,61	+	+	+	PA	1	c			
2020	17c	Organic sunflower oil	+	+M	-	<i>Cronobacter</i> spp.	29	31,77	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,2	+	+	+	PA	1	c			
2020	18c	Manganese sulfate	-										-	/	/	/	/	/	/	-	NA	1	c			
2020	19c	Sinc sulfate	+	+M	-	<i>Cronobacter</i> spp.	29	32,08	+	+	+	PA	+M	-	<i>Cronobacter</i> spp.	26	31,06	+	+	+	PA	1	c			
2020	20c	Biotin	-										-	/	/	/	/	/	/	-	NA	1	c			

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																						
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*				Alternative method: RAPID'Sakazakii													Category	Type
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement – 18 h at 37°C± 1°C																		
				RAPID'Sakazakii (direct streaking 10 µl)																		
				Confirmation																		
CCI	Oxidase	Identification	Result	Typical colonies	Without purification step						With purification step		Negative samples (Subculture CSB + streaking onto CCI)	All confirmatory tests	Final result	Agreement						
Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)			Oxidase	Biochemical gallery (API ID32)																
		Target Cq	I.C. Cq				Final result															
2019	7720	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	st	/	/	-	+p	-	Cronobacter spp.	22,57	32,74	+	-	Cronobacter spp.	/	+	+	PD	2	a	
2019	7721	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	+p	-	+	+	+p	-	Cronobacter spp.	23,05	32,56	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7722	Infant cereals without probiotic 5 cereals	Céréales infantiles sans probiotique 5 céréales	+p	-	+	+	+p	-	Cronobacter spp.	23,26	32,23	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7723	Infant cereals without probiotic vanilla (10 months+)	Céréales infantiles sans probiotique babivanille (10+ mois)	+p	-	+	+	+p	-	Cronobacter spp.	24,24	32,07	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7724	Infant cereals without probiotic cocoa biscuit	Céréales infantiles sans probiotique chocobiscuité	+p	-	+	+	+p	-	Cronobacter spp.	22,84	33,54	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7725	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	+p	-	+	+	+p	-	Cronobacter spp.	22,56	33,38	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7726	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	+p	-	+	+	+p	-	Cronobacter spp.	23,74	35,5	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7727	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	+p	-	+	+	+p	-	Cronobacter spp.	24,00	33,57	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7728	Infant formula stage 2 organic	Poudre de lait infantile bio 2ème âge sans probiotique	+p	-	+	+	+p	-	Cronobacter spp.	23,9	32,03	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7729	Follow-up infant formula	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	+p	-	+	+	+p	-	Cronobacter spp.	24,25	34,75	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7730	Infant formula stage 1	Poudre de lait infantile sans probiotique premier âge	+p	-	+	+	+p	-	Cronobacter spp.	24,59	39,67	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	7731	Infant formula stage 2	Poudre de lait infantile sans probiotique 2ème âge	+p	-	+	+	+p	-	Cronobacter spp.	23,68	32,76	+	-	Cronobacter spp.	/	+	+	PA	2	a	
2019	8011	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8012	Infant formula stage 2	Poudre de lait infantile bio 2ème âge sans probiotique	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8013	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8014	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8015	Follow-up infant formula	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8016	Infant cereals without probiotic vanilla (10 months+)	Céréales infantiles sans probiotique babivanille (10+ mois)	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8017	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	

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

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																						
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*				Alternative method: RAPID'Sakazakii													Category	Type
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement – 18 h at 37°C± 1°C																		
				RAPID'Sakazakii (direct streaking 10 µl)																		
				CCI	Oxidase	Identification	Result	Typical colonies	Confirmation									Negative samples (Subculture CSB + streaking onto CCI)	All confirmatory tests	Final result		
Without purification step									With purification step													
Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)							Oxidase	Biochemical gallery (API ID32)												
		Target Cq	I.C. Cq	Final result																		
2019	8018	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	a	
2019	8019	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	st	/	/	-	+p	-	<i>Cronobacter</i> spp.	22,47	33,01	+	-	<i>Cronobacter</i> spp.	/	+	+	PD	2	a	
2019	8020	Infant cereals without probiotic 5 cereals	Céréales infantiles sans probiotique 5 céréales	st	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	2	a	
2019	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	-	+	+	+p	-	<i>Cronobacter</i> spp.	23,03	32,28	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	/	/	-	+p	-	<i>Cronobacter</i> spp.	23,66	32,04	+	-	<i>Cronobacter</i> spp.	/	+	+	PD	2	b	
2019	7734	Infant formula with probiotics thick formula stage 2 (B.infantis 3,7.10 ⁶ CFU/g)	Poudre de lait avec probiotiques actigest formule épaissie 2ème âge (B.infantis 8,3.10 ⁴ CFU/g)	+p	-	+	+	+p	-	<i>Cronobacter</i> spp.	23,97	32,88	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	/	/	-	+p	-	<i>Cronobacter</i> spp.	23,25	33,17	+	-	<i>Cronobacter</i> spp.	/	+	+	PD	2	b	
2019	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 (Bifidobacteries 1,5.10 ³ CFU/g)	+p	-	+	+	+p	-	<i>Cronobacter</i> spp.	23,38	33,15	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	/	/	-	-	/	/	/	/	/	/	/	-	-	-	NA	2	b	
2019	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	-	+	+	+p	-	<i>Cronobacter</i> spp.	19,52	34,12	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	-	+	+	+p	-	<i>Cronobacter</i> spp.	19,56	34,08	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	-	+	+	+p	-	<i>Cronobacter</i> spp.	21,33	32,05	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	-	+	+	+p	-	<i>Cronobacter</i> spp.	19,83	33,73	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	
2019	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	-	+	+	+p	-	<i>Cronobacter</i> spp.	19,81	34,66	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	b	

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																								
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*				Alternative method: RAPID'Sakazakii										Negative samples (Subculture CSB + streaking onto CCI)	All confirmatory tests	Final result	Agreement	Category	Type	
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement – 18 h at 37°C± 1°C																				
				RAPID'Sakazakii (direct streaking 10 µl)										Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)	Oxidase							Biochemical gallery (API ID32)
								Confirmation																
CCI	Oxidase	Identification	Result	Typical colonies	Without purification step			With purification step																
					Oxidase	Biochemical gallery (API ID32)	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)													
2019	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	-	+	+	+p	-	Cronobacter spp.	18,71	39,49	+	-	Cronobacter spp.	/	+	+	PA	2	b			
2019	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)	-	/	/	-	-	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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)	-	/	/	-	-	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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)	-	/	/	-	-	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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)	-	/	/	-	-	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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)	-	/	/	-	-	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	8109	Infant formula with probiotics thick formula stage 2 (Bifidobacteria 1,5.10 ⁶ CFU/g)	Poudre de lait avec probiotiques formule épaissie 2ème âge (Bifidobacteries 1,5.10 ⁶ CFU/g)	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	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	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	8113	Infant formula with probiotics stage 2 (Bifidobacteria 3,2.10 ⁴ CFU/g)	Poudre de lait avec probiotiques 2ème âge (Bifidobacteries 3,2.10 ⁴ CFU/g)	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	b			
2019	7744	Maltodextrin	Maltodextrine	st	/	/	-	+p	-	Cronobacter spp.	21,09	32,12	+	-	Cronobacter spp.	/	+	+	PD	2	c			
2019	7745	Maltodextrin	Maltodextrine	st	/	/	-	+p	-	Cronobacter spp.	20,91	31,88	+	-	Cronobacter spp.	/	+	+	PD	2	c			
2019	7746	Non-fat dry milk powder	Poudre de lait écrémé	+p	-	+	+	+p	-	Cronobacter spp.	20,73	31,22	+	-	Cronobacter spp.	/	+	+	PA	2	c			
2019	7747	Non-fat dry milk powder	Poudre de lait écrémé	+p	-	+	+	+p	-	Cronobacter spp.	18,91	34,56	+	-	Cronobacter spp.	/	+	+	PA	2	c			
2019	7748	Whey	Lactosérum	st	/	/	-	st	/	/	/	/	/	/	/	st	-	-	NA	2	c			

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																											
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*				Alternative method: RAPID'Sakazakii										Negative samples (Subculture CSB + streaking onto CCI)	All confirmatory tests	Final result	Agreement	Category	Type				
				375g + 1125 mL pre-warmed (37°C) BPW with PIF supplement – 18 h at 37°C± 1°C																							
				RAPID'Sakazakii (direct streaking 10 µl)										Typical colonies	Oxidase	Biochemical gallery (API ID32)	PCR on colony (without FDRS/APF Fast)							Oxidase	Biochemical gallery (API ID32)		
				Without purification step					With purification step								Target Cq									I.C. Cq	Final result
CCI	Oxidase	Identification	Result	Typical colonies	Oxidase	Biochemical gallery (API ID32)	Target Cq	I.C. Cq	Final result	Oxidase	Biochemical gallery (API ID32)																
2019	7749	Whey	Lactoserum	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	7750	Whey	Lactoserum	+p	-	+	+	+p	-	<i>Cronobacter</i> spp.	19,53	32,8	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	c						
2019	7751	Maltodextrin	Maltodextrine	+p	-	+	+	+p	-	<i>Cronobacter</i> spp.	21,08	31,74	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	c						
2019	7752	Whey	Lactoserum	st	/	/	-	+p	-	<i>Cronobacter</i> spp.	20,12	30,94	+	-	<i>Cronobacter</i> spp.	/	+	+	PD	2	c						
2019	7753	Non-fat dry milk powder	Poudre de lait écrémé	+p	-	+	+	+p	-	<i>Cronobacter</i> spp.	19,63	31,85	+	-	<i>Cronobacter</i> spp.	/	+	+	PA	2	c						
2019	7754	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	+p	-	<i>Cronobacter</i> spp.	19,63	32,51	+	-	<i>Cronobacter</i> spp.	/	+	+	PD	2	c						
2019	7755	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	+p	-	<i>Cronobacter</i> spp.	19,95	32,04	+	-	<i>Cronobacter</i> spp.	/	+	+	PD	2	c						
2019	8021	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8022	Maltodextrin	Maltodextrine	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8023	Whey	Lactoserum	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8024	Whey	Lactoserum	st	/	/	-	-	/	/	/	/	/	/	/	/	-	-	-	NA	2	c					
2019	8025	Whey	Lactoserum	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8114	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8115	Non-fat dry milk powder	Poudre de lait écrémé	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8116	Milk powder ingredient	Ingredient poudre de lait	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8117	Milk powder ingredient	Ingredient poudre de lait	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					
2019	8118	Maltodextrin	Maltodextrine	st	/	/	-	st	/	/	/	/	/	/	/	/	st	-	-	NA	2	c					

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																								
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*	Alternative method: RAPID'Sakazakii																	Category	Type	
					375 g + 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										375 g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C ± 1°C + plates storage for 72h at 5°C ±3°C									
					RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Agreement	RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Agreement				
					Result	Typical colonies	Confirmation						Typical colonies	Confirmation										
							Without purification step							Without purification step										
Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)		Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)																		
		Target Cq	I.C. Cq	Final result			Target Cq	I.C. Cq	Final result															
2019	7720	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	-	+p	-	Cronobacter spp.	20,16	36,11	+	+	+	PD	+p	-	Cronobacter spp.	19,46	34,13	+	+	+	PD	2	a
2019	7721	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	+	+p	-	Cronobacter spp.	20,24	34,87	+	+	+	PA	+p	-	NI/ Cronobacter spp.	21,62	32,27	+	+	+	PA	2	a
2019	7722	Infant cereals without probiotic 5 cereals	Céréales infantiles sans probiotique 5 céréales	+	+p	-	NI/ Cronobacter spp.	19,1	36,04	+	+	+	PA	+p	-	NI/ Cronobacter spp.	19,15	33,42	+	+	+	PA	2	a
2019	7723	Infant cereals without probiotic vanilla (10 months+)	Céréales infantiles sans probiotique babivanille (10+ mois)	+	+p	-	Cronobacter spp.	20,52	34,73	+	+	+	PA	+p	-	Cronobacter spp.	19,78	33,46	+	+	+	PA	2	a
2019	7724	Infant cereals without probiotic cocoa biscuit	Céréales infantiles sans probiotique chocobiscuité	+	+p	-	Cronobacter spp.	19,62	35,6	+	+	+	PA	+p	-	Cronobacter spp.	18,79	34,05	+	+	+	PA	2	a
2019	7725	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	+	+p	-	Cronobacter spp.	19,91	34,37	+	+	+	PA	+p	-	Cronobacter spp.	19,22	34,56	+	+	+	PA	2	a
2019	7726	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	+	+p	-	Cronobacter spp.	19,28	33,28	+	+	+	PA	+p	-	Cronobacter spp.	20,89	31,96	+	+	+	PA	2	a
2019	7727	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	+	+p	-	Cronobacter spp.	20,05	33,53	+	+	+	PA	+p	-	NI/ Cronobacter spp..	20,96	33,66	+	+	+	PA	2	a
2019	7728	Infant formula stage 2 organic	Poudre de lait infantile bio 2ème âge sans probiotique	+	+p	-	Cronobacter spp.	19,4	35,17	+	+	+	PA	+p	-	Cronobacter spp.	20,27	32,21	+	+	+	PA	2	a
2019	7729	Infant formula without probiotic	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	+	+p	-	NI/ Cronobacter spp..	19,73	33,12	+	+	+	PA	+p	-	Cronobacter spp.	20,73	31,84	+	+	+	PA	2	a
2019	7730	Infant formula stage 1	Poudre de lait infantile sans probiotique premier âge	+	+p	-	Cronobacter spp.	18,54	41,18	+	+	+	PA	+p	-	Cronobacter spp.	20,07	32,81	+	+	+	PA	2	a
2019	7731	Infant formula stage 2	Poudre de lait infantile sans probiotique 2ème âge	+	+p	-	Cronobacter spp.	19,27	34,53	+	+	+	PA	+p	-	Cronobacter spp.	18,45	32,96	+	+	+	PA	2	a
2019	8011	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	-										st	/	/	/	/	/	-	-	NA	2	a
2019	8012	Infant formula stage 2 organic	Poudre de lait infantile bio 2ème âge sans probiotique	-										st	/	/	/	/	/	-	-	NA	2	a

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

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Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*	Alternative method: RAPID'Sakazakii																		Category	Type					
					375 g + 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									375 g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C ± 1°C + plates storage for 72h at 5°C ±3°C															
					RAPID'Sakazakii (direct streaking 10 µl)									RAPID'Sakazakii (direct streaking 10 µl)															
					Result	Typical colonies	Confirmation							All confirmatory tests	Final result	Agreement	Typical colonies	Confirmation							All confirmatory tests	Final result	Agreement		
							Without purification step											Without purification step											
Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)					Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)																				
		Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq			Final result																				
2019	8013	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	-										st	/	/	/	/	/	-	-	NA	2	a					
2019	8014	Infant formula stage 2	Poudre de lait infantile 2ème âge sans probiotique	-										st	/	/	/	/	/	-	-	NA	2	a					
2019	8015	Follow-up infant formula	Poudre de lait infantile sans probiotique lait de suite bio 2ème âge	-										st	/	/	/	/	/	-	-	NA	2	a					
2019	8016	Infant cereals without probiotic vanilla (10 months+)	Céréales infantiles sans probiotique babivanille (10+ mois)	-										st	/	/	/	/	/	-	-	NA	2	a					
2019	8017	Infant cereals without probiotic vanilla	Céréales infantiles sans probiotique vanille gourmande	-										st	/	/	/	/	/	-	-	NA	2	a					
2019	8018	Infant cereals without probiotic brioche	Céréales infantiles sans probiotique saveur briochée	-										st	/	/	/	/	/	-	-	NA	2	a					
2019	8019	Infant cereals without probiotic multi cereals	Céréales infantiles sans probiotique multicéréales	-	+p	-	<i>Cronobacter</i> spp.	21,31	34,07	+	+	+	PD	+p	-	<i>Cronobacter</i> spp.	22,47	34,46	+	+	+	PD	2	a					
2019	8020	Infant cereals without probiotic 5 cereals	Céréales infantiles sans probiotique 5 céréales	-										-	/	/	/	/	/	-	-	NA	2	a					
2019	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	-	<i>Cronobacter</i> spp.	21,21	32,32	+	+	+	PA	+p	-	NI/ <i>Cronobacter</i> spp.	20,38	32,6	+	+	+	PA	2	b					
2019	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)	-	+p	-	<i>Cronobacter</i> spp.	19,39	33,47	+	+	+	PD	+p	-	NI/ <i>Cronobacter</i> spp.	20,51	32,82	+	+	+	PD	2	b					
2019	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	-	<i>Cronobacter</i> spp.	27,77	31,57	+	+	+	PA	+p	-	NI/ <i>Cronobacter</i> spp.	23,32	31,18	+	+	+	PA	2	b					
2019	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)	-	+p	-	<i>Cronobacter</i> spp.	19,46	-	+	+	+	PD	+p	-	<i>Cronobacter</i> spp.	21,19	37,69	+	+	+	PD	2	b					
2019	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 (Bifidobacteries 1,5.10 ³ CFU/g)	+	+p	-	<i>Cronobacter</i> spp.	20,19	-	+	+	+	PA	+p	-	<i>Cronobacter</i> spp.	19,79	35,81	+	+	+	PA	2	b					

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					375 g + 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										375 g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C ± 1°C + plates storage for 72h at 5°C ±3°C									
					RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Agreement	RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Agreement				
					Result	Typical colonies	Confirmation Without purification step						Typical colonies	Oxi-dase	Biochemical gallery (APID ID32)	Confirmation Without purification step								
Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)	Oxi-dase	Biochemical gallery (APID ID32)			PCR on colony (without FDRS/APF Fast)																	
2019	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)	-										-	/	/	/	/	/	-	-	NA	2	b
2019	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	-	Cronobacter spp.	21,53	32,33	+	+	+	PA	+p	-	Cronobacter spp.	20,67	39,21	+	+	+	PA	2	b
2019	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	-	Cronobacter spp.	24,89	31,91	+	+	+	PA	+p	-	Cronobacter spp.	21,31	34,32	+	+	+	PA	2	b
2019	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	-	Cronobacter spp.	22,03	33,38	+	+	+	PA	+p	-	Cronobacter spp.	23,57	31,66	+	+	+	PA	2	b
2019	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	-	Cronobacter spp.	21,25	34,4	+	+	+	PA	+p	-	Cronobacter spp.	19,52	37,7	+	+	+	PA	2	b
2019	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	-	Cronobacter spp.	23,11	32,3	+	+	+	PA	+p	-	Cronobacter spp.	19,77	37,96	+	+	+	PA	2	b
2019	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	-	Cronobacter spp.	22,12	32,54	+	+	+	PA	+p	-	Cronobacter spp.	20,6	35,32	+	+	+	PA	2	b
2019	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)	-										-	/	/	/	/	/	-	-	NA	2	b
2019	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)	-										-	/	/	/	/	/	-	-	NA	2	b
2019	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)	-										-	/	/	/	/	/	-	-	NA	2	b
2019	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)	-										-	/	/	/	/	/	-	-	NA	2	b

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																								
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*	Alternative method: RAPID'Sakazakii																	Category	Type	
					375 g + 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										375 g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C ± 1°C + plates storage for 72h at 5°C ± 3°C									
					RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Agreement	RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Agreement				
					Result	Typical colonies	Confirmation Without purification step						Typical colonies	Confirmation Without purification step										
							Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)					Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)								
		Target Cq	I.C. Cq	Final result		Target Cq	I.C. Cq	Final result																
2019	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)	-										-	/	/	/	/	/	-	-	NA	2	b
2019	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 (Bifidobacteries 1,5.10 ⁶ CFU/g)	-										st	/	/	/	/	/	-	-	NA	2	b
2019	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	/	/	/	/	/	-	-	NA	2	b
2019	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	/	/	/	/	/	-	-	NA	2	b
2019	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	/	/	/	/	/	-	-	NA	2	b
2019	8113	Infant formula with probiotics stage 2 (Bifidobacteria 3,2.10 ⁴ CFU/g)	Poudre de lait avec probiotiques 2ème âge (Bifidobacteries 3,2.10 ⁴ CFU/g)	-										st	/	/	/	/	/	-	-	NA	2	b
2019	7744	Maltodextrin	Maltodextrine	-	+p	-	NI/ Cronobacter spp.	22,19	32,17	+	+	+	PD	+p	-	Cronobacter spp.	21,49	33,01	+	+	+	PD	2	c
2019	7745	Maltodextrin	Maltodextrine	-	+p	-	Cronobacter spp.	21,03	32,56	+	+	+	PD	+p	-	Cronobacter spp.	21,34	32,75	+	+	+	PD	2	c
2019	7746	Non-fat dry milk powder	Poudre de lait écrémé	+	+p	-	NI/ Cronobacter spp.	22,98	31,93	+	+	+	PA	+p	-	Cronobacter spp.	20,67	32,69	+	+	+	PA	2	c
2019	7747	Non-fat dry milk powder	Poudre de lait écrémé	+	+p	-	Cronobacter spp.	22,34	32,08	+	+	+	PA	+p	-	Cronobacter spp.	18,68	35,31	+	+	+	PA	2	c
2019	7748	Whey	Lactoserum	-										st	/	/	/	/	/	-	-	NA	2	c
2019	7749	Whey	Lactoserum	-										st	/	/	/	/	/	-	-	NA	2	c
2019	7750	Whey	Lactoserum	+	+p	-	Cronobacter spp.	22,29	32,02	+	+	+	PA	+p	-	Cronobacter spp.	19,56	33,78	+	+	+	PA	2	c
2019	7751	Maltodextrin	Maltodextrine	+	+p	-	Cronobacter spp.	20,86	33,04	+	+	+	PA	+p	-	Cronobacter spp.	22,41	32,11	+	+	+	PA	2	c
2019	7752	Whey	Lactoserum	-	+p	-	NI/ Cronobacter spp.	21,1	32,4	+	+	+	PD	+p	-	Cronobacter spp.	20,69	32,76	+	+	+	PD	2	c

INFANT FORMULA AND INFANT CEREALS WITH AND WITHOUT PROBIOTICS INCLUDING INGREDIENTS (375 g sample size) - Study realised by ADRIA																																	
Year of analysis	Sample N°	Product	Product (French name)	Reference method: ISO 22964*	Alternative method: RAPID'Sakazakii																		Category	Type									
					375 g + 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									375 g + 1125 mL pre-warmed (37°C) BPW with PIF supplement - 18h at 37°C ± 1°C + plates storage for 72h at 5°C ±3°C																			
					RAPID'Sakazakii (direct streaking 10 µl)									RAPID'Sakazakii (direct streaking 10 µl)																			
					Result	Typical colonies	Confirmation									All confirmatory tests	Final result	Agreement	Typical colonies	Confirmation									All confirmatory tests	Final result	Agreement		
							Without purification step													Without purification step													
Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)					Oxi-dase	Biochemical gallery (APID ID32)	PCR on colony (without FDRS/APF Fast)																								
		Target Cq	I.C. Cq	Final result	Target Cq	I.C. Cq			Final result																								
2019	7753	Non-fat dry milk powder	Poudre de lait écrémé	+	+p	-	<i>Cronobacter</i> spp.	21,58	33,29	+	+	+	PA	+p	-	<i>Cronobacter</i> spp.	19,31	33,46	+	+	+	PA	2	c									
2019	7754	Non-fat dry milk powder	Poudre de lait écrémé	-	+p	-	NI/ <i>Cronobacter</i> spp.	22,48	32,11	+	+	+	PD	+p	-	<i>Cronobacter</i> spp.	21,71	32,22	+	+	+	PD	2	c									
2019	7755	Non-fat dry milk powder	Poudre de lait écrémé	-	+p	-	NI/ <i>Cronobacter</i> spp.	21,05	32,9	+	+	+	PD	+p	-	<i>Cronobacter</i> spp.	20,15	33,75	+	+	+	PD	2	c									
2019	8021	Non-fat dry milk powder	Poudre de lait écrémé	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8022	Maltodextrin	Maltodextrine	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8023	Whey	Lactoserum	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8024	Whey	Lactoserum	-										-	/	/	/	/	/	-	-	NA	2	c									
2019	8025	Whey	Lactoserum	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8114	Non-fat dry milk powder	Poudre de lait écrémé	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8115	Non-fat dry milk powder	Poudre de lait écrémé	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8116	Milk powder ingredient	Ingredient poudre de lait	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8117	Milk powder ingredient	Ingredient poudre de lait	-										st	/	/	/	/	/	-	-	NA	2	c									
2019	8118	Maltodextrin	Maltodextrine	-										st	/	/	/	/	/	-	-	NA	2	c									

Appendix 5 – Relative level of detection study: raw data

Matrix: Infant cereals with probiotics - 30g

Study realized by Q-Laboratories Inc

Strain: *Cronobacter sakazakii* QL 11007.9

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

Unpaired

Lactic acid bacteria: 2,4.10³ CFU/g

Level	Inoculation level (cfu/25g)	Sample N° (reference method)	ISO 22964				Sample N° (alternative method)	Alternative method: RAPID'Sakazakii- 30 g + 270 mL BPW - 16h at 37 ± 1°C									
			CCI	Oxidase	Identification	Result		Positive Results/Total (ISO22964)	RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	RAPID'Sakazakii		
									Typical colonies	Oxidase	Biochemical gallery (API ID32)	Confirmatory tests			Final result	Positive results/Total	
												PCR on colony (APF Fast without FDRS)					
										Target Cq	I.C. Cq	Final result					
0	0	8	-	/	/	-	0/5	3	-	/	/	/	/	/	-	-	
		10	-	/	/	-		16	-	/	/	/	/	/	-	-	
		12	-	/	/	-		18	-	/	/	/	/	/	-	-	
		16	-	/	/	-		23	-	/	/	/	/	/	-	-	
		27	-	/	/	-		24	-	/	/	/	/	/	-	-	
Low	1	1	-	/	/	-	7/20	1	-	/	/	/	/	/	-	-	
		2	-	/	/	-		2	+M	-	<i>Cronobacter</i> spp.	26,57	31,29	+	+	+	
		4	-	/	/	-		4	+M	-	<i>Cronobacter</i> spp.	26,82	32,3	+	+	+	
		5	+M	-	<i>Cronobacter</i> spp.	+		5	+M	-	<i>Cronobacter</i> spp.	26,35	30,8	+	+	+	
		6	-	/	/	-		6	-	/	/	/	/	/	-	-	
		7	-	/	/	-		7	+M	-	<i>Cronobacter</i> spp.	27,04	31,28	+	+	+	
		9	-	/	/	-		9	-	/	/	/	/	/	-	-	
		11	+M	-	<i>Cronobacter</i> spp.	+		10	+M	-	<i>Cronobacter</i> spp.	26,44	30,67	+	+	+	
		13	+M	-	<i>Cronobacter</i> spp.	+		11	-	/	/	/	/	/	-	-	
		14	-	/	/	-		12	+M	-	<i>Cronobacter</i> spp.	26,43	31,13	+	+	+	
		15	-	/	/	-		13	-	/	/	/	/	/	-	-	
		17	+M	-	<i>Cronobacter</i> spp.	+		14	-	/	/	/	/	/	-	-	
		18	-	/	/	-		15	-	/	/	/	/	/	-	-	
		19	-	/	/	-		19	-	/	/	/	/	/	-	-	
		20	+M	-	<i>Cronobacter</i> spp.	+		21	-	/	/	/	/	/	-	-	
		21	-	/	/	-		25	+M	-	<i>Cronobacter</i> spp.	26,38	30,75	+	+	+	
		25	-	/	/	-		26	+M	-	<i>Cronobacter</i> spp.	26,27	31,01	+	+	+	
		26	+M	-	<i>Cronobacter</i> spp.	+		27	-	/	/	/	/	/	-	-	
		29	-	/	/	-		28	-	/	/	/	/	/	-	-	
		30	+M	-	<i>Cronobacter</i> spp.	+		29	-	/	/	/	/	/	-	-	
High	8	3	+M	-	<i>Cronobacter</i> spp.	+	5/5	8	+M	-	<i>Cronobacter</i> spp.	26,59	31,01	+	+	+	
		22	+M	-	<i>Cronobacter</i> spp.	+		17	+M	-	<i>Cronobacter</i> spp.	26,73	31,28	+	+	+	
		23	+M	-	<i>Cronobacter</i> spp.	+		20	+M	-	<i>Cronobacter</i> spp.	26,73	31,28	+	+	+	
		24	+M	-	<i>Cronobacter</i> spp.	+		22	+M	-	<i>Cronobacter</i> spp.	26,28	31,11	+	+	+	
		28	+M	-	<i>Cronobacter</i> spp.	+		30	+M	-	<i>Cronobacter</i> spp.	26,26	30,07	+	+	+	

Matrix : Powdered infant formula with probiotics - 375g

Strain : *Cronobacter sakazakii* Ad1418

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

Unpaired

Lactic count: 1,7.10⁶ CFU/g

Sample N°	Level	Inoculation level (cfu/10g)	Reference method: ISO 22964♦					Alternative method: RAPID'Sakazakii 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)	RAPID'Sakazakii (direct streaking 10 µl)					All confirmatory tests	Final result	Positive Results/Total	
								Typical colonies	Oxidase	Biochemical gallery (API ID32)	PCR on colony (APF Fast without FDRS)					
											Target Cq	I.C. Cq				Final result
8205	0	/	st	/	/	-	0 /5	st	/	/	/	/	/	-	-	0 /5
8206			st	/	/	-		st	/	/	/	/	/	-	-	
8207			st	/	/	-		st	/	/	/	/	/	-	-	
8208			st	/	/	-		st	/	/	/	/	/	-	-	
8209			st	/	/	-		st	/	/	/	/	/	-	-	
8268	Low	1,9	st	/	/	-	14/20	+p	-	<i>Cronobacter</i> spp.	23,17	32,30	+	+	+	15/20
8269			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,96	31,53	+	+	+	
8270			st	/	/	-		+p	-	<i>Cronobacter</i> spp.	23,35	36,40	+	+	+	
8271			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,78	32,70	+	+	+	
8272			+p	-	+	+		st	/	/	/	/	/	-	-	
8273			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,54	32,62	+	+	+	
8274			st	/	/	-		+p	-	<i>Cronobacter</i> spp.	23,79	34,70	+	+	+	
8275			st	/	/	-		+p	-	<i>Cronobacter</i> spp.	23,12	33,49	+	+	+	
8276			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,58	32,79	+	+	+	
8277			st	/	/	-		+d/+	-	<i>Cronobacter</i> spp.	23,09	32,17	+	+	+	
8278			+p	-	+	+		st	/	/	/	/	/	-	-	
8279			st	/	/	-		st	/	/	/	/	/	-	-	
8280			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	24,13	32,93	+	+	+	
8281			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,77	32,95	+	+	+	
8282			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,97	31,09	+	+	+	
8283			+p	-	+	+		st	/	/	/	/	/	-	-	
8284			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	24,13	31,76	+	+	+	
8285			+p	-	+	+		st	/	/	/	/	/	-	-	
8286			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,96	32,12	+	+	+	
8287			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	24,40	33,35	+	+	+	
8288	High	5,1	+p	-	+	+	5 /5	+p	-	<i>Cronobacter</i> spp.	23,96	33,50	+	+	+	5 /5
8289			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,67	32,22	+	+	+	
8290			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	24,09	35,82	+	+	+	
8291			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	24,42	31,25	+	+	+	
8292			+p	-	+	+		+p	-	<i>Cronobacter</i> spp.	23,65	32,82	+	+	+	

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

Appendix 6 – Inclusivity and exclusivity study: raw data

INCLUSIVITY (Initial validation study, 2012)							
No	Genus	Species	Reference	Origin	Inoculation level CFU/ 225ml BPW	RAPID'Sakazakii	Confirmation (oxidase, biochemical tests)
1.	<i>Cronobacter</i>	<i>dublinensis</i>	DSM18705	Dairy Product	2	+	+
2.	<i>Cronobacter</i>	<i>malonaticus</i>	DSM18702	Dairy Product	22	+	+
3.	<i>Cronobacter</i>	<i>malonaticus</i>	Ad 1708	Dairy product	19	+	+
4.	<i>Cronobacter</i>	<i>muytjensii</i>	CIP103581	/	1	+	+
5.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 939	Infant formula	3	+	+
6.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 940	Infant formula	1	+	+
7.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 941	Infant formula	1	+	+
8.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 942	Infant formula	7	+	+
9.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 943	Infant formula	5	+	+
10.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 944	Infant formula	2	+	+
11.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 945	Infant formula	4	+	+
12.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 946	Infant formula	1	+	+
13.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 947	Infant formula	4	+	+
14.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 948	Infant formula	31	+	+
15.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 949	Infant formula	3	+	+
16.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 950	Infant formula	4	+	+
17.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 951	Infant formula	1	+	+
18.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 952	Infant formula	6	+	+
19.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 953	Infant formula	4	+	+
20.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 963	Infant formula	2	+	+
21.	<i>Cronobacter</i>	<i>sakazakii</i>	95	Infant formula	8	+	+
22.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 704	Infant formula	3	+	+
23.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 831	Infant formula	4	+	+

INCLUSIVITY (Initial validation study, 2012)							
No	Genus	Species	Reference	Origin	Inoculation level CFU/ 225ml BPW	RAPID'Sakazakii	Confirmation (oxidase, biochemical tests)
24.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 829	Infant formula	5	+	+
25.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 916	Infant formula	2	+	+
26.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 893	Infant formula	6	+	+
27.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 894	Infant formula	8	+	+
28.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 895	Infant formula	6	+	+
29.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 896	Infant formula	2	+	+
30.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 897	Infant formula	4	+	+
31.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 898	Infant formula	56	+	+
32.	<i>Cronobacter</i>	<i>dublinensis lactaridi</i>	DSMZ18707 T	Dairy Product	18	+	+
33.	<i>Cronobacter</i>	<i>dublinensis lausannensis</i>	DSMZ 18706 T	Dairy Product	12	+	+
34.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1418	Infant formula	5	+	+
35.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1419	Infant formula	16	+	+
36.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1420	Infant formula	4	+	+
37.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1421	Infant formula	8	+	+
38.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1424	Infant formula	7	+	+
39.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1425	Infant formula	3	+	+
40.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1426	Infant formula	11	+	+
41.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1427	Infant formula	12	+	+
42.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1428	Infant formula	4	+	+
43.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1429	Infant formula	3	+	+
44.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1430	Infant formula	4	+	+
45.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1431	Infant formula	10	+	+
46.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1432	Infant formula	5	+	+
47.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1433	Infant formula	17	+	+
48.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1434	Infant formula	6	+	+

INCLUSIVITY (Initial validation study, 2012)							
No	Genus	Species	Reference	Origin	Inoculation level CFU/ 225ml BPW	RAPID'Sakazakii	Confirmation (oxidase, biochemical tests)
49.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1435	Infant formula	4	+	+
50.	<i>Cronobacter</i>	<i>sakazakii</i>	Ad 1436	Infant formula	4	+	+
51.	<i>Cronobacter</i>	<i>turicensis</i>	Ad 1445	Infant formula	7	+	+
52.	<i>Cronobacter</i>	<i>turicensis</i>	DSMZ 18703	/	4	+	+

INCLUSIVITY (ADRIA, 2019)													
Strains						RAPID'Sakazakii - (37°C) BPW + specific supplement (d¼). Incubation for 18 h at 37°C ± 1°C							
No	Genus	Species	N°	Origin	Inoculation Level (CFU/225ml BPW)	Confirmation		PCR on colonies					
						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
1	<i>Cronobacter</i>	<i>dublinensis</i>	DSM18705	Dairy Product	12	+p	+	20,6	34,12	+	21,69	34,8	+
2	<i>Cronobacter</i>	<i>malonaticus</i>	DSM18702	Dairy Product	2	+p	+	21,24	32,14	+	21,04	32,34	+
3	<i>Cronobacter</i>	<i>malonaticus</i>	Ad1708	Dairy Product	21	+p	+	20,27	33,22	+	20,4	33,93	+
4	<i>Cronobacter</i>	<i>muytjensii</i>	CIP103581	/	6	+p	+	16,16	-	+	18,19	-	+
5	<i>Cronobacter</i>	<i>sakazakii</i>	Ad939	Infant formula	14	+p	+	19,86	32,39	+	19,86	33,67	+
6	<i>Cronobacter</i>	<i>sakazakii</i>	Ad940	Infant formula	53	+p	+	18,66	-	+	19,33	39,99	+
7	<i>Cronobacter</i>	<i>sakazakii</i>	Ad941	Infant formula	22	+p	+	18,18	-	+	19,47	40,56	+
8	<i>Cronobacter</i>	<i>sakazakii</i>	Ad942	Infant formula	27	+p	+	20,27	33,08	+	20,58	35,04	+
9	<i>Cronobacter</i>	<i>sakazakii</i>	Ad943	Infant formula	10	+p	+	25,76	31,62	+	16,27	31,71	+
10	<i>Cronobacter</i>	<i>sakazakii</i>	Ad944	Infant formula	20	+p	+	21,6	33,17	+	21,75	34,09	+
11	<i>Cronobacter</i>	<i>sakazakii</i>	Ad945	Infant formula	14	+p	+	19,36	33,85	+	26,09	-	+
12	<i>Cronobacter</i>	<i>sakazakii</i>	Ad946	Infant formula	14	+p	+	20,15	44,01	+	21,45	36,75	+
13	<i>Cronobacter</i>	<i>sakazakii</i>	Ad947	Infant formula	20	+p	+	19,91	32,13	+	28,7	35,81	+
14	<i>Cronobacter</i>	<i>sakazakii</i>	Ad948	Infant formula	24	+p	+	19,19	33,5	+	22,99	31,7	+
15	<i>Cronobacter</i>	<i>sakazakii</i>	Ad949	Infant formula	8	+p	+	23,35	36,44	+	24,34	33,12	+
16	<i>Cronobacter</i>	<i>sakazakii</i>	Ad950	Infant formula	19	+p	+	23,31	31,52	+	23,78	31,54	+
17	<i>Cronobacter</i>	<i>sakazakii</i>	Ad951	Infant formula	14	+p	+	21,7	-	+	23,63	32,53	+
18	<i>Cronobacter</i>	<i>sakazakii</i>	Ad952	Infant formula	24	+p	+	19,5	32,84	+	18,65	35,28	+
19	<i>Cronobacter</i>	<i>sakazakii</i>	Ad953	Infant formula	32	+p	+	21,48	32,93	+	22,8	32,69	+
20	<i>Cronobacter</i>	<i>sakazakii</i>	Ad963	Infant formula	19	+p	+	20,75	36,17	+	21,72	42,42	+

INCLUSIVITY (ADRIA, 2019)													
Strains						RAPID'Sakazakii - (37°C) BPW + specific supplement (d¼). Incubation for 18 h at 37°C ± 1°C							
No	Genus	Species	N°	Origin	Inoculation Level (CFU/225ml BPW)	Confirmation		PCR on colonies					
						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
21	<i>Cronobacter</i>	<i>sakazakii</i>	95	Infant formula	21	+p	+	22,38	32,05	+	21,63	32,27	+
22	<i>Cronobacter</i>	<i>sakazakii</i>	Ad704	Infant formula	26	+p	+	17,63	-	+	20,38	33,97	+
23	<i>Cronobacter</i>	<i>sakazakii</i>	Ad831	Infant formula	26	+p	+	20,84	32,57	+	20,49	33,96	+
24	<i>Cronobacter</i>	<i>sakazakii</i>	Ad829	Infant formula	16	+p	+	19,48	34,63	+	19,56	38,28	+
25	<i>Cronobacter</i>	<i>sakazakii</i>	Ad916	Infant formula	15	+p	+	19,19	-	+	21,05	33,09	+
26	<i>Cronobacter</i>	<i>sakazakii</i>	Ad893	Infant formula	17	+p	+	19,7	34,13	+	20,1	33,13	+
27	<i>Cronobacter</i>	<i>sakazakii</i>	Ad894	Infant formula	14	+p	+	19,38	34,67	+	19,95	36,22	+
28	<i>Cronobacter</i>	<i>sakazakii</i>	Ad895	Infant formula	7	+p	+	19,02	-	+	18,35	-	+
29	<i>Cronobacter</i>	<i>sakazakii</i>	Ad896	Infant formula	13	+p	+	19,87	32,58	+	19,51	35,85	+
30	<i>Cronobacter</i>	<i>sakazakii</i>	Ad897	Infant formula	21	+p	+	19,44	36,76	+	19,58	36,79	+
31	<i>Cronobacter</i>	<i>sakazakii</i>	Ad898	Infant formula	9	+p	+	19,97	32,88	+	20,22	33,94	+
32	<i>Cronobacter</i>	<i>dublinensis lactaridi</i>	DSMZ18707 T	Dairy Product	12	+p	+	21,21	32,2	+	21,49	31,9	+
33	<i>Cronobacter</i>	<i>dublinensis lausannensis</i>	DSMZ 18706 T	Dairy Product	23	+p	+	21,52	33,1	+	19,76	36,52	+
34	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1418	Infant formula	17	+p	+	21,24	32,03	+	21,28	32,29	+
35	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1419	Infant formula	24	+p	+	20,41	31,66	+	21,03	32,08	+
36	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1420	Infant formula	14	+p	+	18,49	37,31	+	18,12	38,45	+
37	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1421	Infant formula	12	+p	+	20,14	33,01	+	20,05	32,95	+
38	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1424	Infant formula	16	+p	+	18,36	38,13	+	18,22	41,11	+
39	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1425	Infant formula	11	+p	+	20,01	33,8	+	19,58	40,55	+

INCLUSIVITY (ADRIA, 2019)													
Strains						RAPID'Sakazakii - (37°C) BPW + specific supplement (d¼). Incubation for 18 h at 37°C ± 1°C							
No	Genus	Species	N°	Origin	Inoculation Level (CFU/ 225ml BPW)	Confirmation		PCR on colonies					
						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
40	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1426	Infant formula	26	+p	+	20,8	32,04	+	21,27	31,48	+
41	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1427	Infant formula	33	+p	+	16,89	-	+	17,35	-	+
42	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1428	Infant formula	13	+p	+	23,5	32,41	+	23,4	32,04	+
43	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1429	Infant formula	12	+p	+	22,11	31,99	+	22,31	31,47	+
44	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1430	Infant formula	27	+p	+	21,19	31,81	+	21,27	31,62	+
45	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1431	Infant formula	21	+p	+	20,21	31,78	+	20,96	32,38	+
46	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1432	Infant formula	16	+p	+	20,92	33,28	+	21,14	34,21	+
47	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1433	Infant formula	19	+p	+	21,53	32,76	+	20,05	34,98	+
48	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1434	Infant formula	18	+p	+	22,51	32,72	+	20,24	36,59	+
49	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1435	Infant formula	15	+p	+	23,18	34,1	+	19,61	-	+
50	<i>Cronobacter</i>	<i>sakazakii</i>	Ad1436	Infant formula	9	+p	+	19,37	34,79	+	19,55	34,32	+
51	<i>Cronobacter</i>	<i>turicensis</i>	Ad 1445	Infant formula	20	+p	+	20,98	31,21	+	20,26	33,46	+
52	<i>Cronobacter</i>	<i>turicensis</i>	DSMZ 18703	/	20	+p	+	19,29	33,45	+	20,45	32,48	+

EXCLUSIVITY (Initial validation study, 2012)						
No	Genus	Species	Reference	Origin	Inoculation level CFU/ml BPW	RAPID'Sakazakii
1	<i>Citrobacter</i>	<i>braakii</i>	Ad833	Beef	3.90E+05	Clear blue colonies
2	<i>Citrobacter</i>	<i>diversus</i>	Ad173	Dairy product	1.70E+05	-
3	<i>Citrobacter</i>	<i>fameri</i>	Ad116	Environmental sample	3.10E+05	Purple colonies
4	<i>Citrobacter</i>	<i>freundi</i>	39	Environmental sample	2.20E+05	Purple-blue colonies
5	<i>Citrobacter</i>	<i>koseri</i>	CIP105177		2.50E+05	-
6	<i>Enterobacter</i>	<i>aerogenes</i>	Ad889	Meat flour	1.70E+05	-
7	<i>Enterobacter</i>	<i>agglomerans</i>	11	Dairy product	2.60E+05	Few pale purple colonies
8	<i>Enterobacter</i>	<i>agglomerans</i>	136	Dairy product	1.00E+03	-
9	<i>Enterobacter</i>	<i>amnigenus</i>	52	Vegetables	8.00E+03	-
10	<i>Enterobacter</i>	<i>amnigenus</i>	129	Raw milk	3.60E+05	-
11	<i>Enterobacter</i>	<i>amnigenus</i>	A00C068	Poultry	1.40E+05	-
12	<i>Enterobacter</i>	<i>cloacae</i>	51	Vegetables	5.60E+05	-
13	<i>Enterobacter</i>	<i>cloacae</i>	10	Dairy product	3.00E+05	-
14	<i>Enterobacter</i>	<i>fergusonii</i>	2876	Environmental sample	3.90E+05	Pale purple colonies
15	<i>Enterobacter</i>	<i>gergoviae</i>	CIP76.1	/	2.00E+05	-
16	<i>Enterobacter</i>	<i>hormaechei</i>	Ad990	Butter	3.00E+05	Pale purple colonies
17	<i>Enterobacter</i>	<i>intermedius</i>	60	Vegetables	1.80E+05	-
18	<i>Enterobacter</i>	<i>kobei</i>	Ad706	Milk powder	2.40E+05	-
19	<i>Escherichia</i>	<i>coli</i>	16	Dairy product	5.70E+05	Clear blue colonies
20	<i>Escherichia</i>	<i>hermanii</i>	Ad462	Dairy product	2.20E+05	Clear blue micro-colonies
21	<i>Hafnia</i>	<i>alvei</i>	Ad245	Dairy product	8.30E+04	-
22	<i>Klebsiella</i>	<i>pneumoniae</i>	122	Dairy product	9.50E+04	Clear blue colonies
23	<i>Kluyvera</i>	<i>oxytoca</i>	MI030497b	Milk powder	2.10E+05	Clear blue colonies
24	<i>Leclercia</i>	<i>adecarboxylata</i>	Ad707	Milk powder	9.80E+04	-
25	<i>Salmonella</i>	<i>diarizonae</i> SIIIb 65 :c :z	Ad 1298	Dairy environmental sample	2.20E+05	Purple colonies
26	<i>Salmonella</i>	Typhimurium	Ad1333	Dairy product	5.80E+05	Purple colonies
27	<i>Serratia</i>	<i>ficaria</i>	113	Vegetables	4.20E+05	Clear blue colonies
28	<i>Serratia</i>	<i>marcescens</i>	Ad455	Raw milk	5.80E+05	-
29	<i>Salmonella</i>	<i>arizonae</i> (51:z4,z23)	CIP 5523	/	4.80E+05	-
30	<i>Yersinia</i>	<i>intermediae</i>	Ad133	Dairy product	2.20E+05	-
31	<i>Enterobacter</i>	<i>helveticus</i>	DSM 18396 T	Fruit powder	4.20E+04	Pale blue micro-colonies

**Appendix 7 – Inter-laboratory study: results obtained by the collaborative laboratories and the expert laboratory
(Initial validation study, 2012)**

Laboratory A
Aerobic mesophilic flora: 66/ml

N°Sample	Reference method ISO / TS 22964					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
A2	-	/	/	/	-	-	/	/	/	-	NA
A6	-	/	/	/	-	-	/	/	/	-	NA
A10	-	/	/	/	-	-	/	/	/	-	NA
A11	-	/	/	/	-	-	/	/	/	-	NA
A17	-	/	/	/	-	-	/	/	/	-	NA
A19	-	/	/	/	-	-	/	/	/	-	NA
A22	-	/	/	/	-	-	/	/	/	-	NA
A24	-	/	/	/	-	-	/	/	/	-	NA
A1	+	+	-	+	+	+	+	-	+	+	PA
A4	-	/	/	/	-	-	/	/	/	-	NA
A9	-	/	/	/	-	-	/	/	/	-	NA
A12	-	/	/	/	-	-	/	/	/	-	NA
A14	-	/	/	/	-	-	/	/	/	-	NA
A18	-	/	/	/	-	-	/	/	/	-	NA
A20	-	/	/	/	-	-	/	/	/	-	NA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
B2	-	/	/	/	-	-	/	/	/	-	NA
B6	-	/	/	/	-	-	/	/	/	-	NA
B10	-	/	/	/	-	-	/	/	/	-	NA
B11	-	/	/	/	-	-	/	/	/	-	NA
B17	-	/	/	/	-	-	/	/	/	-	NA
B19	-	/	/	/	-	-	/	/	/	-	NA
B22	-	/	/	/	-	-	/	/	/	-	NA
B24	-	/	/	/	-	-	/	/	/	-	NA
B1	-	/	/	/	-	-	/	/	/	-	NA
B4	-	/	/	/	-	-	/	/	/	-	NA
B9	-	/	/	/	-	-	/	/	/	-	NA
B12	-	/	/	/	-	-	/	/	/	-	NA
B14	-	/	/	/	-	-	/	/	/	-	NA
B18	-	/	/	/	-	-	/	/	/	-	NA
B20	+	+	-	+	+	+	+	-	+	+	PA
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 0,1ml into mLST+V

N°Sample	Reference method ISO / TS 22964					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
C2	-	/	/	/	-	-	/	/	/	-	NA
C6	+	+	-	+	+	-	/	/	/	-	ND
C10	-	/	/	/	-	-	/	/	/	-	NA
C11	-	/	/	/	-	-	/	/	/	-	NA
C17	-	/	/	/	-	-	/	/	/	-	NA
C19	-	/	/	/	-	-	/	/	/	-	NA
C22	+	+	-	+	+	+	+	-	+	+	PA
C24	-	/	/	/	-	-	/	/	/	-	NA
C1	+	+	-	+	+	+	+	-	+	+	PA
C4	+	+	-	+	+	+	+	-	+	+	PA
C9	+	+	-	+	+	+	+	-	+	+	PA
C12	+	+	+	-	+	+	+	-	+	+	PA
C14	+	+	+	-	+	+	+	-	+	+	PA
C18	-	/	/	/	-	-	/	/	/	-	NA
C20	-	/	/	/	-	-	/	/	/	-	NA
C21	-	/	/	/	-	-	/	/	/	-	NA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
D2	-	/	/	/	-	-	/	/	/	-	NA
D6	-	/	/	/	-	-	/	/	/	-	NA
D10	-	/	/	/	-	-	/	/	/	-	NA
D11	-	/	/	/	-	-	/	/	/	-	NA
D17	-	/	/	/	-	-	/	/	/	-	NA
D19	-	/	/	/	-	-	/	/	/	-	NA
D22	-	/	/	/	-	-	/	/	/	-	NA
D24	-	/	/	/	-	-	/	/	/	-	NA
D1	+	+	-	+	+	+	+	-	+	+	PA
D4	-	/	/	/	-	-	/	/	/	-	NA
D9	+	+	-	+	+	+	+	-	+	+	PA
D12	+	+	-	+	+	+	+	-	+	+	PA
D14	+	+	-	+	+	+	+	-	+	+	PA
D18	-	/	/	/	-	-	/	/	/	-	NA
D20	-	/	/	/	-	-	/	/	/	-	NA
D21	-	/	/	/	-	-	/	/	/	-	NA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
E2	-	/	/	/	-	-	/	/	/	-	NA
E6	-	/	/	/	-	-	/	/	/	-	NA
E10	-	/	/	/	-	-	/	/	/	-	NA
E11	-	/	/	/	-	-	/	/	/	-	NA
E17	-	/	/	/	-	-	/	/	/	-	NA
E19	-	/	/	/	-	-	/	/	/	-	NA
E22	-	/	/	/	-	-	/	/	/	-	NA
E24	-	/	/	/	-	-	/	/	/	-	NA
E1	+	+	-	+	+	+	+	-	+	+	PA
E4	-	/	/	/	-	-	/	/	/	-	NA
E9	+	+	-	+	+	+	+	-	+	+	PA
E12	+	+	-	+	+	+	+	-	+	+	PA
E14	+	+	-	+	+	+	+	-	+	+	PA
E18	+	+	-	+	+	+	+	-	+	+	PA
E20	+	+	-	+	+	+	+	-	+	+	PA
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 0,1ml into mLST+V

N°Sample	Reference method ISO / TS 22964					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
F2	-	/	/	/	-	-	/	/	/	-	NA
F6	-	/	/	/	-	-	/	/	/	-	NA
F10	-	/	/	/	-	-	/	/	/	-	NA
F11	-	/	/	/	-	-	/	/	/	-	NA
F17	-	/	/	/	-	-	/	/	/	-	NA
F19	-	/	/	/	-	-	/	/	/	-	NA
F22	-	/	/	/	-	-	/	/	/	-	NA
F24	+	+	-	+	+	-	/	/	/	-	ND
F1	+	+	-	+	+	+	+	-	+	+	PA
F4	+	+	-	+	+	+	+	-	+	+	PA
F9	+	+	-	+	+	+	+	-	+	+	PA
F12	-	/	/	/	-	-	/	/	/	-	NA
F14	+	+	-	+	+	+	+	-	+	+	PA
F18	+	+	-	+	+	-	/	/	/	-	ND
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 0,1ml into mLST+V

N°Sample	Reference method ISO / TS 22964					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
G2	-	/	/	/	-	-	/	/	/	-	NA
G6	-	/	/	/	-	-	/	/	/	-	NA
G10	-	/	/	/	-	-	/	/	/	-	NA
G11	-	/	/	/	-	-	/	/	/	-	NA
G17	+	+	-	+	+	+	+	-	+	+	PA
G19	-	/	/	/	-	-	/	/	/	-	NA
G22	+	+	-	+	+	+	+	-	+	+	PA
G24	-	/	/	/	-	-	/	/	/	-	NA
G1	-	/	/	/	-	-	/	/	/	-	NA
G4	+	+	-	+	+	+	+	-	+	+	PA
G9	-	/	/	/	-	-	/	/	/	-	NA
G12	-	/	/	/	-	-	/	/	/	-	NA
G14	+	+	-	+	+	-	/	/	/	-	ND
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 0,1ml into mLST+V

N°Sample	Reference method ISO / TS 22964					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
H2	-	/	/	/	-	-	/	/	/	-	NA
H6	+	+	-	+	+	+	+	-	+	+	PA
H10	-	/	/	/	-	-	/	/	/	-	NA
H11	+	+	-	+	+	-	/	/	/	-	ND
H17	-	/	/	/	-	+	+	-	+	+	PD
H19	+	+	-	+	+	-	/	/	/	-	ND
H22	+1col	+	-	+	-	+	+	-	+	+	PD
H24	-	/	/	/	-	-	/	/	/	-	NA
H1	+	+	-	+	+	+	+	-	+	+	PA
H4	-	/	/	/	-	-	/	/	/	-	NA
H9	-	/	/	/	-	+	+	-	+	+	PD
H12	+	+	-	+	+	+	+	-	+	+	PA
H14	+	+	-	+	+	+	+	-	+	+	PA
H18	+	+	-	+	+	+ 1 col	+	-	+	+	PA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
I2	-	/	/	/	-	-	/	/	/	-	NA
I6	-	/	/	/	-	-	/	/	/	-	NA
I10	-	/	/	/	-	-	/	/	/	-	NA
I11	-	/	/	/	-	-	/	/	/	-	NA
I17	-	/	/	/	-	-	/	/	/	-	NA
I19	-	/	/	/	-	-	/	/	/	-	NA
I22	-	/	/	/	-	-	/	/	/	-	NA
I24	-	/	/	/	-	-	/	/	/	-	NA
I1	+	+	-	+	+	+	+	-	+	+	PA
I4	-	/	/	/	-	-	/	/	/	-	NA
I9	+	+	-	+	+	+	+	-	+	+	PA
I12	-	/	/	/	-	-	/	/	/	-	NA
I14	+	+	-	+	+	+	+	-	+	+	PA
I18	+	+	-	+	+	+	+	-	+	+	PA
I20	-	/	/	/	-	-	/	/	/	-	NA
I21	+	+	-	+	+	+	+	-	+	+	PA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
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	+	+	-	+	+	+	+	-	+	+	PA
J20	+	+	-	+	+	+	+	-	+	+	PA
J21	-	/	/	/	-	-	/	/	/	-	NA
J3	+	+	-	+	+	+	+	-	+	+	PA
J5	+	+	-	+	+	+	+	-	+	+	PA
J7	+	+	-	+	+	+	+	-	+	+	PA
J8	+	+	-	+	+	+	+	-	+	+	PA
J13	+	+	-	+	+	+	+	-	+	+	PA
J15	+	+	-	+	+	+	+	-	+	+	PA
J16	+	+	-	+	+	+	+	-	+	+	PA
J23	+	+	-	+	+	+	+	-	+	+	PA

Laboratory **K**
 Aerobic mesophilic flora:2,0 10⁵ cfu/ml

N°Sample	Reference method ISO / TS 22964					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
K2	-	/	/	/	-	-	/	/	/	-	NA
K6	-	/	/	/	-	-	/	/	/	-	NA
K10	-	/	/	/	-	-	/	/	/	-	NA
K11	-	/	/	/	-	-	/	/	/	-	NA
K17	-	/	/	/	-	-	/	/	/	-	NA
K19	-	/	/	/	-	-	/	/	/	-	NA
K22	-	/	/	/	-	-	/	/	/	-	NA
K24	-	/	/	/	-	-	/	/	/	-	NA
K1	-	/	/	/	-	-	/	/	/	-	NA
K4	-	/	/	/	-	-	/	/	/	-	NA
K9	-	/	/	/	-	-	/	/	/	-	NA
K12	+	+	-	+	+	+	+	-	+	+	PA
K14	-	/	/	/	-	-	/	/	/	-	NA
K18	+	+	-	+	+	+	+	-	+	+	PA
K20	-	/	/	/	-	-	/	/	/	-	NA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
L2	-	/	/	/	-	-	/	/	/	-	NA
L6	-	/	/	/	-	-	/	/	/	-	NA
L10	-	/	/	/	-	-	/	/	/	-	NA
L11	-	/	/	/	-	-	/	/	/	-	NA
L17	-	/	/	/	-	-	/	/	/	-	NA
L19	-	/	/	/	-	-	/	/	/	-	NA
L22	-	/	/	/	-	-	/	/	/	-	NA
L24	-	/	/	/	-	-	/	/	/	-	NA
L1	-	/	/	/	-	-	/	/	/	-	NA
L4	-	/	/	/	-	-	/	/	/	-	NA
L9	-	/	/	/	-	-	/	/	/	-	NA
L12	+	+	-	+	+	+	+	-	+	+	PA
L14	+	+	-	+	+	+	+	-	+	+	PA
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:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation		Final result	RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E			TCS	Oxidase	API 20E		
M2	-	/	/	/	-	-	/	/	/	-	NA
M6	-	/	/	/	-	-	/	/	/	-	NA
M10	-	/	/	/	-	+	+	-	+	+	PD
M11	-	/	/	/	-	-	/	/	/	-	NA
M17	-	/	/	/	-	-	/	/	/	-	NA
M19	-	/	/	/	-	-	/	/	/	-	NA
M22	-	/	/	/	-	-	/	/	/	-	NA
M24	-	/	/	/	-	-	/	/	/	-	NA
M1	-	/	/	/	-	-	/	/	/	-	NA
M4	-	/	/	/	-	-	/	/	/	-	NA
M9	-	/	/	/	-	-	/	/	/	-	NA
M12	+	+	-	+	+	+	+	-	+	+	PA
M14	+	+	-	+	+	+	+	-	+	+	PA
M18	+	+	-	+	+	+	+	-	+	+	PA
M20	-	/	/	/	-	-	/	/	/	-	NA
M21	-	/	/	/	-	-	/	/	/	-	NA
M3	+	+	-	+	+	+	+	-	+	+	PA
M5	+	+	-	+	+	+	+	-	+	+	PA
M7	+	+	-	+	+	+	+	-	+	+	PA
M8	+	+	-	+	+	+	+	-	+	+	PA
M13	+	+	-	+	+	+	+	-	+	+	PA
M15	+	+	-	+	+	+	+	-	+	+	PA
M16	+	+	-	+	+	+	+	-	+	+	PA
M23	+	+	-	+	+	+	+	-	+	+	PA

Laboratory **N (ADRIA)**
 Aerobic mesophilic flora:145cfu/ml

N°Sample	Reference method ISO / TS 22964 ♦					Alternative method:RAPID'Sakazakii					Agreement
	Typical colonies		Confirmation			RAPID'Sakazakii	Confirmation			Final result	
	COMPASS <i>Enterobacter sakazakii</i>	TCS	Oxidase	API 20E	Final result		TCS	Oxidase	API 20E		
N2	-	/	/	/	-	-	/	/	/	-	NA
N6	-	/	/	/	-	-	/	/	/	-	NA
N10	-	/	/	/	-	-	/	/	/	-	NA
N11	-	/	/	/	-	-	/	/	/	-	NA
N17	-	/	/	/	-	-	/	/	/	-	NA
N19	-	/	/	/	-	-	/	/	/	-	NA
N22	-	/	/	/	-	-	/	/	/	-	NA
N24	-	/	/	/	-	-	/	/	/	-	NA
N1	+	+	-	+	+	+	+	-	+	+	PA
N4	+	+	-	+	+	+	+	-	+	+	PA
N9	-	/	/	/	-	-	/	/	/	-	NA
N12	+	+	-	+	+	+	+	-	+	+	PA
N14	-	/	/	/	-	-	/	/	/	-	NA
N18	-	/	/	/	-	-	/	/	/	-	NA
N20	+	+	-	+	+	+	+	-	+	+	PA
N21	-	/	/	/	-	-	/	/	/	-	NA
N3	+	+	-	+	+	+	+	-	+	+	PA
N5	+	+	-	+	+	+	+	-	+	+	PA
N7	+	+	-	+	+	+	+	-	+	+	PA
N8	+	+	-	+	+	+	+	-	+	+	PA
N13	+	+	-	+	+	+	+	-	+	+	PA
N15	+	+	-	+	+	+	+	-	+	+	PA
N16	+	+	-	+	+	+	+	-	+	+	PA
N23	+	+	-	+	+	+	+	-	+	+	PA

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