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Summary Report

for the ISO 16140-2 validation of the iQ-Check EB for the detection of *Enterobacteriaceae* in infant formula, infant cereals with or without probiotics and related ingredients

MicroVal study number	**2018LR80**
Method/Kit names	iQ-Check EB for the detection of *Enterobacteriaceae*
Report version	**Summary Report (MCS & ILS)** - **Version 2** 02 September 2025
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Standardized report - Qualitative methods

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LIST OF ABBREVIATIONS

Method & protocol

CFU	Colony Forming Units
DW	Deep Well
IC	Internal Control
ILS	Interlaboratory Study
MCS	Method Comparison Study
MVTC	MicroVal Technical Committee
RLOD	Relative Level of Detection
RT	Relative Trueness
SE	Relative Sensitivity
SP	Relative Specificity

Interpretation

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

Raw data

-	No typical colonies but presence of background microflora
-A	No typical colonies but presence of high level of background microflora
-B	No typical colonies but presence of medium level of background microflora
-C	No typical colonies but presence of low level of background microflora
(x)	Number of colonies in the plate
*	Dilution of the extract in case of inhibition according to the alternative protocol (1/10)
1/2	50% level of target analyte
d	Doubtful result
i	Inhibition
m	Minority level of target analyte
M	Majority level of target analyte
p	Pure culture level of target analyte
st	Plate without any colony
Bold typing	**Artificially inoculated samples**

Modifications are highlighted.

Foreword

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

Validation protocol	▪ ISO 16140-1 (2016): Microbiology of the food chain - Method validation — *Part 1: Vocabulary* ▪ ISO 16140-2 (2016) & ISO 16140-2/A1 (2024): Microbiology of the food chain - Method validation — *Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method* ▪ MicroVal technical committee interpretation of ISO 16140-2 v.2.6
Reference method♦	ISO 21528-1 (2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection of *Enterobacteriaceae*
Method/Kit name	**iQ-Check *Enterobacteriaceae* (EB)**
Scope of validation	**Infant formula, infant cereals with and without probiotics and related ingredients (up to 375 g test portion)**
Certification organization	LRQA

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

1 INTRODUCTION

This document reports a MicroVal validation study, based on ISO 16140-2:2016 and ISO 16140-2/A1 (2024) of alternative method (iQ-Check *Enterobacteriaceae* (EB)) method for detection of *Enterobacteriaceae* in infant formula, infant cereals with or without probiotics and related ingredients (up to 375 g test portions) carried out by ADRIA as the MicroVal Expert laboratory.

The following kits and instrument are concerned by the validation study:

- For extraction and PCR: the iQ-Check *Enterobacteriaceae* kit (Catalog #12003068)
- FDRS (catalog# 3594970)
- PCR instruments:
    - CFX96 Deep Well (catalog# 3600037)
    - CFX Opus Deep Well (catalog# 12016658)

The protocol is the following:

- Enrichment step: 1/4 prewarmed BPW broth (1125ml) + PIF supplement for 18 - 26 h at 37°C ± 1°C.
- Enriched sample can be tested directly or diluted 1/10 in BPW prior to lysis.
- FDRS treatment.
- Easy I lysis protocol on 100 µL FDRS treated sample.
- PCR on 5 µL lysate using the CFX96 Deep Well and CFX Opus Deepwell.

The confirmation of positive PCR results is performed as follows:

- Reference method protocol: streaking 10 µl of the primary enriched broth onto VRBG plate, incubate for 24 h ± 2 h at 37°C ± 1°C. Oxidase and OF tests are used to confirm the colonies.
- Alternative method protocol: streaking 10 µl of the primary enriched broth onto RAPID *Enterobacteriaceae* agar. The only presence of typical colonies allows to confirm the positive PCR result.

The reference method used is: ISO 21528-1:2017: Microbiology of the food chain – Horizontal method for the detection of *Enterobacteriaceae* – Part 1: detection of *Enterobacteriaceae.*

The category included in the scope is: infant cereals, infant formula with or without probiotics and related ingredients (up to 375 g test portion size).

In June 2025, an extension for the use of a new version of the CFX Maestro IDE software (v4.0) was approved. The data are available upon request from the MicroVal technical committee.

2 METHOD PROTOCOLS

2.1 Reference method♦

See the flow diagram in **Appendix 1**.

The reference method used for this study corresponds to the ISO 21528-1 (2017): Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection of *Enterobacteriaceae.*

2.2 Alternative method

See the flow diagram of the alternative method in **Appendix 2**.
See the kit insert in **Appendix 3**.

2.2.1 Principle

The iQ-Check *Enterobacteriaceae* real-time PCR assay is based on gene amplification and detection. The kit's ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for EB, as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with a Bio-Rad real-time PCR instrument, such as the CFX96 Deep Well and the CFX Opus Deepwell with the CFX Manager IDE software.

The iQ-Check Free DNA Removal Solution, provided in a separate kit, is used with the iQ-Check *Enterobacteriaceae* real-time PCR kit to optimize removal of free DNA.

The iQ-Check *Enterobacteriaceae* real-time PCR kit is provided in a ready-to-use format containing all primers, probes, and reagents (except for template DNA) required for the PCR reaction. In addition, an internal positive control (IPC) is included in the reaction mix to identify possible PCR inhibition.

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

2.2.2 Protocol

Samples were prepared according to some of the requirement of the ISO 6887 as described in the table below.

Table 1 – Sample preparation

Matrix	ISO 21528-1	iQ-Check EB	Sample preparation according to
Products with more than 20% fat	1/10 BPW + Tween 80 (10 g/L)	1/4 BPW + PIF	ISO 6887-1 ISO 6887-4
Infant cereals (blowing agent)	1/10 BPW + alpha-amylase (0.1 g/l)	1/4 BPW + PIF + alpha-amylase (0.1 g/l)	ISO 6887-4
Products with probiotics	1/10 BPW 2T or BPW + vancomycin (10mg/L)	1/4 BPW + PIF	ISO 6887-4 ISO 6887-5

The claimed protocol tested for the study was the following:

- Up to 375g test portion
- Enrichment step: 1/4 prewarmed BPW broth (1125ml) + PIF supplement for 18 - 26 h at 37°C ± 1°C.
- Enriched sample was tested directly (protocol ①) and after 1/10 dilution in BPW (protocol ② - *not tested during the ILS*).
- Easy I lysis protocol on 100 µL FDRS treated sample.
- PCR on 5 µL lysate using the CFX96 Deep Well and CFX Opus Deepwell.

The confirmation of positive PCR results is as follows:

- Following ISO 21528-1 confirmation protocol using VRBG plates
- Alternative method protocol:
    - streaking 10 µl of the primary enriched broth onto RAPID *Enterobacteriaceae* agar. The only presence of typical colonies allows to confirm the positive PCR result.

It is possible to store the enrichment broths for 72 h at 5°C ± 3°C before proceeding to PCR and confirmatory tests.

2.2.3 Restriction

There is no restriction.

2.3 Study design

The study is **an unpaired study design** as the reference and the alternative methods have different enrichment procedures and test portions.

50 g portions were used for the alternative method with the protocol ① for the sample preparation while 10 g test portions were used for the reference method.

3 METHOD COMPARISON STUDY

3.1 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 Categories and sample types

One category was included in this validation study. A minimum of 60 items for each category were tested by both the reference method and the alternative method in the sensitivity study, with a minimum of 30 positive samples per category.
Each category was made up of 3 types, with at least 20 items representative for that Type.
64 samples were analysed. The distribution of positive and negative samples per tested category and type is given respectively in Table 2.

Table 2 – Distribution per tested category and type

Category		Test portion size (g)	Alternative method protocol	Type		Positive samples	Negative samples	Total
1	**Infant formula, infant cereals with or without probiotics and related ingredients**	375	Protocol ①	**a**	Infant formula and infant cereals without probiotics	14	7	21
				b	Infant formula and infant cereals with probiotics	15	5	20
				c	Ingredients including milk powder	15	8	23
					Total	**44**	**20**	**64**
		375	Protocol ②	**a**	Infant formula and infant cereals without probiotics	15	6	21
				b	Infant formula and infant cereals with probiotics	13	7	20
				c	Ingredients including milk powder	15	8	23
					Total	**43**	**21**	**64**

3.1.2 Test sample preparation

Artificial contaminations were done by seeding protocols with lyophilised strain and storage 1 or 2 weeks at ambient temperature.

The artificial contaminations are presented in **Appendix 4**.

The same strain was not used to inoculate more than 6 samples.

4 samples were artificially contaminated by *seeding*, using 11 different strains and a seeding protocol. 37 gave a positive result with the protocol ① and 36 with protocol ②. For one sample (1921), another strain than the one inoculated were recovered, this sample was therefore considered as naturally contaminated.

Most of the seeding inoculations were lower or equal to 5 CFU/sample.

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	Seeding protocol		Total positive samples
			≤ 3 CFU	3 < x ≤ 5.6 CFU	
Protocol ①	1	7	35	2	44
	%	15.9	79.5	4.5	100.0
Protocol ②	1	7	34	2	43
	%	15.9	79.5	4.5	100.0

15.7 % of the samples were naturally contaminated with all types represented.

3.1.3 Protocols applied during the validation study

Incubation times

The minimum incubation time was applied for the study: 18h.

Extraction protocol

The Easy I protocol with FDRS was applied to the two protocols (① & ②).

PCR

The APF EB Fast was used with the CFX Opus Deepwell.

In case of inhibition, the DNA extract was 1:10 diluted in distilled water prior to performing the PCR reaction.

Confirmation protocols

The confirmation of positive PCR results was performed as follows:

- Reference method protocol: streaking 10 µl of the enriched broth onto VRBG plate, incubate for 24 h ± 2 h at 37°C ± 1°C. During the validation, Oxidase and OF tests will used to confirm the colonies.
- Alternative method protocol: streaking 100 µl of enriched broth onto RAPID *Enterobacteriaceae* agar. During the validation, Oxidase and OF tests will used to confirm the colonies.

> ***Enrichment broths storage for 72 h at 5 °C ± 3 °C***

The storage of the enrichment broth was tested after 72h incubation time at 5 ± 3°C. PCR tests and confirmatory tests will be performed again.

3.1.4 Sensitivity study results

All raw data per category are given in **Appendix 5**. Sample numbers in **bold** indicate artificial inoculation of the sample (see **Appendix 4** for details on artificial inoculation).

Table 4 shows the summary of results of the reference method and the alternative methods for both protocols.

Table 4 – Summary of sensitivity study results for protocols ① and ②

		Reference method positive (R+)	Reference method negative (R-)
Protocol ①	**Alternative method positive (A+)**	Positive agreement (R+/A+) **PA = 21**	Positive deviation (R-/A+) **PD = 15**
	Alternative method negative (A-)	Negative deviation (R+/A-) **TND = 8**	Negative agreement (R-/A-) **TNA = 20**
Protocol ②	**Alternative method positive (A+)**	Positive agreement (R+/A+) **PA = 21**	Positive deviation (R-/A+) **PD = 14**
	Alternative method negative (A-)	Negative deviation (R+/A-) **TND = 8**	Negative agreement (R-/A-) **TNA = 21**

With $TNA = NA + NA_{FN\ (alt)} + PD_{FP\ (alt)}$ $TND = ND + ND_{FN\ (alt)} + PA_{FP\ (alt)}$

3.1.5 Sensitivity study calculations

The sensitivity study parameters as specified in Table 5 were calculated for all categories and types, and the overview is given in Table 6.

Table 5 – Formula to calculate the sensitivity parameters

		Protocol ①	Protocol ②
Sensitivity for the alternative method	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \; x \; 100\ \%$	81.8%	81.4%
Sensitivity for the reference method	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \; x \; 100\ \%$	65.9%	67.4%
Relative trueness	$RT = \frac{(PA + TNA)}{N} x 100\ \%$	64.1 %	65.6%
False positive ratio for the alternative method	$FPR = \frac{PA_{FP(alt)} + PD_{FP\,(alt)}}{TNA} \; x \; 100\ \%$	10.0%	9.5%
False negative ratio for the alternative method	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	0.07	0.09

With $TNA = NA + NA_{FN\,(alt)} + PD_{FP\,(alt)}$ $TND = ND + ND_{FN\,(alt)} + PA_{FP\,(alt)}$

Table 6 – Overview calculated sensitivity parameters per category and type

Pure enrichment - Easy I lysis with FDRS (Protocol ①)

Category		Types		PA	$PA_{FP(alt)}$	NA	$NA_{FN(alt)}$	PD	ND	$ND_{FN(alt)}$	$PD_{FP(alt)}$	TND	TNA	SE_{alt} %	SE_{ref} %	RT %	FPR %	FNR
1	Infant cereals, infant formula with or without probiotics and ingredients	a	Infant formula and infant cereals without probiotics	8	0	5	1	4	2	0	1	2	7	85.7	71.4	71.4	14.3	0.07
		b	Infant formula and infant cereals with probiotics	6	0	4	0	6	3	0	1	3	5	80.0	60.0	55.0	20.0	0.00
		c	Ingredients including milk powder	7	0	7	1	5	2	1	0	3	8	80.0	66.7	65.2	0.0	0.13
	Total			**21**	**0**	**16**	**2**	**15**	**7**	**1**	**2**	**8**	**20**	**81.8**	**65.9**	**64.1**	**10.0**	**0.07**

1/10 enriched sample - Easy I lysis with FDRS (Protocol ②)

Category		Types		PA	$PA_{FP(alt)}$	NA	$NA_{FN(alt)}$	PD	ND	$ND_{FN(alt)}$	$PD_{FP(alt)}$	TND	TNA	SE_{alt} %	SE_{ref} %	RT %	FPR %	FNR
1	Infant cereals, infant formula with or without probiotics and ingredients	a	Infant formula and infant cereals without probiotics	7	0	6	0	5	2	1	0	3	6	80.0	66.7	61.9	0.0	0.07
		b	Infant formula and infant cereals with probiotics	6	0	3	2	4	3	0	2	3	7	76.9	69.2	65.0	28.6	0.15
		c	Ingredients including milk powder	8	0	7	1	5	2	0	0	2	8	86.7	66.7	69.6	0.0	0.07
	Total			**21**	**0**	**16**	**3**	**14**	**7**	**1**	**2**	**8**	**21**	**81.4**	**67.4**	**65.6**	**9.5**	**0.09**

3.1.6 Discordant results

Negative deviations

Eight negative deviations were observed for both protocols, all coming from artificially contaminated samples. For two samples (1459 whey and 1913 infant cereals), the results were different between the two protocols and the presence of *the Enterobacteriaceae* strain (*Hafnia alvei* and *Lelliotia amnigena)* was confirmed in the enriched broth ($ND_{FN(alt)}$). The DNA extract was tested three times but remained negative. Note that after 72 h storage of the enrichment broth a positive PCR result was obtained for one of the samples (1913, Cq:19.3).

According to ISO 16140-2, samples in negative agreement were also evaluated by the confirmation method. For five of them (3 with protocol ① and 4 with protocol ②), the *Enterobacteriaceae* strain was confirmed in the enriched broth ($NA_{FN(alt)}$ = 5). The DNA extract was tested additional times and for one of them positive results were obtained for some replicates, the other samples remaining negative. The samples are listed Table 7.

Positive deviations

Fifteen positive deviations were obtained for protocol ① and fourteen for protocol ②. They concern all types of products and different types of strain (*Citrobacter*, *Escherichia*, *Cronobacter*, *Klebsiella*, *Hafnia*, *Serratia*). Results are consistent between the two protocols for thirteen samples. Only three samples show differences between the two protocols (1436, 1453 and 1454). Five of them are naturally contaminated samples.

Negative deviations are listed in Table 8 and positive deviations in Table 9.

Table 7 - Negative agreement samples with positive confirmation

Year of analysis	Sample n°	Product	Protocol	iQ-Check *Enterobacteriaceae*					Category	Type
				1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h 37 ± 1°C						
				PCR result - CFX OPUS - Easy I lysis with FDRS			Confirmation	Agreement Ref/Alt		
2024	**1436**	**Infant formula (23.6% fat level)**	①	N/A/20.92/21.14/ 37.99/37.47/N/A/ 36.03/N/A/37.41/ 38.42	32.63/32.03/32.29/ 32.39/32.29/32.55/ 31.98/32.61/32.43/ 32.43	-/+/+/+/+/-/+/-/+/+	+	$NA_{FN(alt)}$	1	a
2024	**1445**	**Caseinate**	①	N/A/N/A/N/A	32.05/32.68/32;51	-/-/-	+	$NA_{FN(alt)}$	1	c
			②	N/A	33.26	-/-/-	+	$NA_{FN(alt)}$		
2024	**1453**	**Infant formula with probiotics (*B. lactis*: 2.7 x 10^3 CFU/g)**	②	N/A/N/A/N/A	34.59/37.82/38.78	-/-/-	+	$NA_{FN(alt)}$	1	b
2024	**1454**	**Infant formula with probiotics (*B. lactis*: 3.9 x10^5 CFU/g)**	②	N/A/N/A/N/A	32.04/36.44/36.13	-/-/-	+	$NA_{FN(alt)}$	1	b

Table 8 - Negative deviations

Year of analysis	Sample N°	Product	Strain inoculated	Inoculation level CFU/sample	ISO method Enrichment broth	ISO 21528-1♦ result	iQ-Check *Enterobacteriaceae*									Category	Type
							1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h 37 ± 1°C										
							PCR result - CFX OPUS							Agreement Ref/Alt			
							Easy I lysis with FDRS			1/10 sample - Easy I lysis with FDRS			Confirmation				
							Cq	IC Cq	Result	Cq	IC Cq	Result		Protocol ①	Protocol ②		
2024	**1437**	**Infant cereals. biscuit taste**	*Cronobacter dublinensis* Ad2848	0.8	BPW + α-amylase	**+**	N/A	34.75	-	N/A	32.85	-	-	**ND**	**ND**	1	a
2024	**1456**	**Infant formula with probiotics (Bifidobacteria: 1.7 x10⁴ CFU/g)**	*Lelliottia amnigena* 129	1	BPW 2X + α-amylase	**+**	N/A	33.05	-	N/A	33.77	-	-	**ND**	**ND**	1	b
2024	**1459**	**Whey**	*Hafnia alvei* Ad2274	<1	BPW	**+**	N/A/N/A/N/A	32.57/ 32.72/ 43.18	-/-/-	34.05	32.2	+	+	**ND$_{FN(alt)}$**	**PA**	1	c
2024	**1913**	**Infant cereals**	*Lelliottia amnigena* 129	3	BPW + α-amylase	**+**	36.33	32.16	+	N/A/N/A/N/A	32.21/ 34.14/ 34.29	-/-/-	+	**PA**	**ND$_{FN(alt)}$**	1	a
2024	**1914**	**Infant cereals**	*Enterobacter kobei* Ad706	1.2	BPW + α-amylase	**+**	N/A	32.59	-	N/A	32.55	-	-	**ND**	**ND**	1	a
2024	**1917**	**Infant formula with probiotics (*Limocilactobacillus*: 6.3 x 10⁶ CFU/g) (>20% fat level)**	*Enterobacter kobei* Ad706	1.2	BPW 2X + Tween 80	**+**	N/A	31.58	-	N/A	32.13	-	-	**ND**	**ND**	1	b
2024	**1918**	**Infant cereals with probiotics (*B. lactis*: 1.2 x 10⁶ CFU/g)**	*Salmonella* Anatum Ad2718	0.5	BPW 2X + Tween 80	**+**	N/A	35.81	-	N/A	33.49	-	-	**ND**	**ND**	1	b
2024	**1919**	**Maltodextrin**	*Serratia macescens* Ad455	0.7	BPW	**+**	N/A	32.27	-	N/A	32.79	-	-	**ND**	**ND**	1	c
2024	1921	Caseinate	*Lelliottia amnigena* 129	3	BPW	**+ (*E. coli*)**	N/A/N/A/N/A	31.76/ 32.19/ 32.30	-/-/-	N/A/N/A/N/A	32.48/ 32.39/ 32.34	-/-/-	- (16S: *Pseudomonas oryzihabitans*)	**ND**	**ND**	1	c

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

Table 9 - Positive deviations

Year of analysis	Sample N°	Product	Strain inoculated	Inoculation level CFU/sample	ISO method Enrichment broth	ISO 21528-1♦ result	iQ-Check *Enterobacteriaceae*						Confirmation	Agreement Ref/Alt		Category	Type
							1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h 37 ± 1°C										
							PCR result - CFX OPUS										
							Easy I lysis with FDRS			1/10 sample - Easy I lysis with FDRS							
							Cq	IC Cq	Result	Cq	IC Cq	Result		Protocol ①	Protocol ②		
2024	**1433**	**Infant formula (23% fat level)**	*Klebsiella pneumoniae* 120	1	BPW + Tween 80	-	23.98	33.25	+	24.32	N/A	+	+	**PD**	**PD**	1	a
2024	**1436**	**Infant formula (23.6% fat level)**	*Serratia macescens* Ad455	<1	BPW + Tween 80	-	N/A/20.92/21.14/ 37.99/37.47/N/A/ 36.03/N/A/37.41/ 38.42	32.63/32.03/32.29/ 32.39/32.29/32.55/ 31.98/32.61/32.43/ 32.43	-/+/+/+/ +/-/+/-/+/+	37.50	33.03	+	+	$NA_{FN(alt)}$	**PD**	1	a
2024	**1438**	**Infant cereals, vegetables**	*Cronobacter dublinensis* Ad2848	0.8	BPW + α-amylase	-	17.76	N/A	+	20.53	36.88	+	+	**PD**	**PD**	1	a
2024	**1439**	**Infant cereals. multicereals, vegetables**	*Cronobacter dublinensis* Ad2848	0.8	BPW + α-amylase	-	37.31	36.46	+	28.23	32.64	+	+	**PD**	**PD**	1	a
2024	**1443**	**Lactose**	*Escherichia hermanii* Ad464	<1	BPW	-	20.06	31.35	+	24.47	33.96	+	+	**PD**	**PD**	1	c
2024	**1444**	**Whey proteins**	*Escherichia hermanii* Ad464	<1	BPW	-	25.39	31.07	+	N/A/N/A/N/A/ 27.43*/ 27.33*	31.95/N/A/ N/A/31.31/ 31.32	i/i/i/+*/+*	+	**PD**	**PD**	1	c
2024	**1448**	**Infant formula with probiotics (*S. thermophilus*: 1.7 x 10^4 CFU/g) (24.3% fat level)**	*Klebsiella pneumoniae* 120	1	BPW 2X + Tween 80	-	28.13	32.03	+	27.39	31.57	+	+	**PD**	**PD**	1	b
2024	**1453**	**Infant formula with probiotics (*B. lactis*: 2.7 x 10^3 CFU/g)**	*Cronobacter dublinensis* Ad2848	0.8	BPW 2X + α-amylase	-	31.43	32.65	+	N/A/N/A/N/A	34.59/37.82/ 38.78	-/-/-	+	**PD**	$NA_{FN(alt)}$	1	b
2024	**1454**	**Infant formula with probiotics (*B. lactis*: 3.9 x 10^5 CFU/g)**	*Cronobacter dublinensis* Ad2848	0.8	BPW 2X + α-amylase	-	31.03	32.35	+	N/A/N/A/N/A	32.04/36.44/ 36.13	-/-/-	+	**PD**	$NA_{FN(alt)}$	1	b
2024	**1455**	**Infant formula with probiotics (Bifidobacteria: 2.6 x 10^4 CFU/g)**	*Cronobacter dublinensis* Ad2848	0.8	BPW 2X + α-amylase	-	21.60	37.31	+	22.90	N/A	+	+	**PD**	**PD**	1	b
2024	**1458**	**Lactose**	*Hafnia alvei* Ad2274	<1	BPW	-	36.94	33.10	+	33.88	32.3	+	+	**PD**	**PD**	1	c
2024	**2231**	Infant cereals. vanilla taste			BPW + α-amylase	-	31.08	34.04	+	19.37	31.81	+	+	**PD**	**PD**	1	a

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

Year of analysis	Sample N°	Product	Strain inoculated	Inoculation level CFU/sample	ISO method Enrichment broth	ISO 21528-1♦ result	iQ-Check *Enterobacteriaceae* 1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h 37 ± 1°C						Confirmation	Agreement Ref/Alt		Category	Type
							PCR result - CFX OPUS										
							Easy I lysis with FDRS			1/10 sample - Easy I lysis with FDRS							
							Cq	IC Cq	Result	Cq	IC Cq	Result		Protocol ①	Protocol ②		
2024	**2232**	Infant cereals with probiotics (Bifidobacteria: 2.6 x 10^4 CFU/g)			BPW 2X + α-amylase	-	22.32	34.16	+	28.19	31.61	+	+ (*Enterobacter cloacae*)	**PD**	**PD**	1	b
2024	**2234**	Infant cereals with probiotics (*B. lactis*: 1.0 x 10^8 CFU/g)			BPW 2X + α-amylase	-	34.35	34	+	23.07	31.76	+	+ (*Enterobacter cloacae*)	**PD**	**PD**	1	b
2024	**2241**	Wheat starch			BPW	-	15.93	38.66	+	17.16	32.96	+	+ (*Enterobacter cloacae*)	**PD**	**PD**	1	c
2024	**2244**	Maltodextrin			BPW	-	27.22	31.53/31.41/31.45	+	36.37	32.98	+	- (+ at 72 h : *Serratia plymuthica*)	**PD**	**PD**	1	c

The analyses of discordant results according to the ISO 16140-2 (2016) and ISO 16140-2/A1(2024) for an unpaired study is the following (See Tables 10 and 11):

Table 10 - Interpretation of the sensitivity study results - Protocol ①

								Unpaired	
Category		**Sample size (g)**	**Types**		**N+**	**TND**	**PD**	**TND - PD**	**AL**
1	Infant cereals, infant formula with or without probiotics and ingredients	375	a	Infant formula and infant cereals without probiotics	14	2	4	-2	
		375	b	Infant formula and infant cereals with probiotics	15	3	6	-3	
		375	c	Ingredients including milk powder	15	3	5	-2	
	Total				**44**	**8**	**15**	**-7**	**3**

Table 11 - Interpretation of the sensitivity study results - Protocol ②

								Unpaired	
Category		**Sample size (g)**	**Types**		**N+**	**TND**	**PD**	**TND - PD**	**AL**
1	Infant cereals, infant formula with or without probiotics and ingredients	375	a	Infant formula and infant cereals without probiotics	15	3	5	-2	
		375	b	Infant formula and infant cereals with probiotics	13	3	4	-1	
		375	c	Ingredients including milk powder	15	2	5	-3	
	Total				**43**	**8**	**14**	**-6**	**3**

The observed values for TND - PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).

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

Primary enrichment broth (BPW+PIF) storage was done at 5°C ± 3°C for 72 h.

49 samples were tested again after enrichment broth storage. Three changes were observed for protocol ① and five for protocol ②. They concerned six samples. They are listed in Table 12.

Table 12 - Observed changes in results before and after storage of the enrichment broth

Category		Sample N°	Product	Before storage					After storage					Category	Type
				PCR results		Final result confirmatory tests	Agreement		PCR results		Final result confirmatory tests	Agreement			
				Protocol ①	Protocol ②		Protocol ①	Protocol ②	Protocol ①	Protocol ②		Protocol ①	Protocol ②		
1	Infant cereals, infant formula with or without probiotics and ingredients	1436	Infant formula (23.6% fat level)	-/+/+/+/+/-/+/-/+/+	+	+	$NA_{FN(alt)}$	PD	+	+	+	PD	PD	1	a
		1444	Whey proteins	+	i/i/i/+*/+*	+	PD	PD	+	-/i/i/+*/+*	+	PD	$NA_{FN(alt)}$	1	c
		1453	Infant formula with probiotics (*B. lactis*: 2.7 x 10^3 CFU/g)	+	-/-/-	+	PD	$NA_{FN(alt)}$	-/-/-	+	+	$NA_{FN(alt)}$	PD	1	b
		1454	Infant formula with probiotics (*B. lactis*: 3.9 x 10^5 CFU/g)	+	-/-/-	+	PD	$NA_{FN(alt)}$	+	+	+	PD	PD	1	b
		1456	Infant formula with probiotics (Bifidobacteria: 1.7 x 10^4 CFU/g)	-	-	-	$ND_{FN(alt)}$	$ND_{FN(alt)}$	+	+	+	PA	PA	1	b
		1913	Infant cereals	+	-/-/-	+	PA	$ND_{FN(alt)}$	+	+	+	PA	PA	1	a

FDRS: iQ-Check Free DNA Removal Solution

The analyses of discordant become (See Tables 13 and 14).

Table 13 - Interpretation of the sensitivity study results after storage of the enrichment broth at 5°C ± 3°C – Protocol ①

								Unpaired	
Category		Sample size (g)	Types		N+	TND	PD	TND-PD	AL
1	Infant cereals, infant formula with or without probiotics and ingredients	375	a	Infant formula and infant cereals without probiotics	15	2	5	-3	
		375	b	Infant formula and infant cereals with probiotics	14	2	5	-3	
		375	c	Ingredients including milk powder	15	3	5	-2	
	Total				44	7	15	-8	3

Table 14 - Interpretation of the sensitivity study results after storage of the enrichment broth at 5°C ± 3°C – protocol ②

								Unpaired	
Category		Sample size (g)	Types		N+	TND	PD	TND - PD	AL
1	Infant cereals, infant formula with or without probiotics and ingredients	375	a	Infant formula and infant cereals without probiotics	15	2	5	-3	
		375	b	Infant formula and infant cereals with probiotics	15	2	6	-4	
		375	c	Ingredients including milk powder	14	2	4	-2	
	Total				44	6	15	-9	3

The observed values for TND - PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).

3.1.8 PCR inhibitions

226 PCR were tested were tested during the validation study, combining before and after storage, and with protocol ① or ②.

Inhibitions were observed two times for one sample (0.9%) and only with protocol ② before and after storage. The dilution of the DNA extract to 1/10 was sufficient to remove the inhibition.

The inhibitions are listed in Table 15.

Table 15 - Sample with inhibited PCR results

Year of analysis	Sample N°	Product	Before or after 72h storage	iQ-Check *Enterobacteriaceae*					
				1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C					
				PCR result - CFX OPUS					
				Protocol ①			Protocol ②		
				Cq	IC Cq	Result	Cq	IC Cq	Result
2024	**1444**	**Whey proteins**	Before	25.39	31.07	+	N/A/N/A/N/A/ 27.43*/27.33*	31.95/N/A/N/A/ 31.31/31.32	i/i/i/+*/+*
			After	25.88	30.82	+	N/A/N/A/N/A/ 27.43*/27.33*	31.95/N/A/N/A/ 31.31/31.32	-/i/i/+*/+*

**: Dilution in distilled water using 10µL of the DNA extract

3.1.9 Confirmation

For this validation, two confirmations were evaluated: streaking on VRBG and on RAPID'*Enterobacteriaceae*. For most of the samples, identical results were found between the two methods.
For three samples (1458, 2234 and 2241), the presence of typical colonies was observed on RAPID *Enterobacteriaceae* while no typical colony appeared on VRBG. For one sample (2232), the opposite was observed.
For two samples (2230 and 2244), the confirmations were negative before storage but positive after 72h at 5 ± 3°C. These samples were naturally contaminated.

Three samples showed positive PCR results unconfirmed by the confirmation method ($PD_{FP(alt)}$=3), two for protocol ① and two for protocol ②. Repetition of the PCR was carried on and fractional results were sometimes obtained. The confirmation was still negative after storage.

3.2 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:

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

3.2.1 Categories, sample types and strains

One (matrix/strain) pair was analyzed by the reference method and by the alternative method (See Table 16).

Table 16 - List of selected types and strains per category, as tested within the relative level of detection study

Category		Matrix	Strain	Origin	Inoculation and storage condition	Enrichment protocol
1	Infant formula, infant cereals with or without probiotics and related ingredients	Infant formula with probiotics	*Enterobacter kobei* Ad706	Milk powder	Seeding protocol Lyophilized strain 2 weeks at room temperature	Prewarmed BPW + PIF 18-26 h at 37°C

3.2.2 Test sample preparations

Three levels of artificial contamination were prepared for each type:

- Negative control level: one non-inoculated in order to get 5 test portions,
- Low level: inoculation at 1.6 CFU/test portion in order to get 20 test portions providing fractional recovery,
- Higher level: inoculation at 4.8 CFU/test portion in order to get 5 test portions contaminated at a higher level.

Test portions were individually inoculated and kept at an appropriate time/temperature for stabilization before actual testing.

3.2.3 RLOD study results

The tabulated raw data on the RLOD are given in **Appendix 6**.

The RLOD calculations were performed using the Excel spreadsheet available at http://standards.iso.org/iso/16140 - RLOD version 4 (2024-01-10). The RLOD are given in Table 17.

Table 17 – Presentation of RLOD before and after confirmation of the alternative method results

Category		Matrix / strain pair	Protocol	AL	RLOD [95% confidence limit]
1	**Infant cereals, infant formula with or without probiotics and ingredients**	Infant formula with probiotics / *Enterobacter Kobei* Ad706	**Protocol ①**	2.5	0.87 [0.43; 1.78]
			Protocol ②	2.5	0,79 [0.39; 1.61]

3.2.4 LOD_{50} results

The LOD_{50} calculations were done using the Excel spreadsheet available at http://standards.iso.org/iso/16140 POD-LOD calculation program - version 12, 2024-03-05. The tests are given in Table 18.

Table 18 - LOD_{50} results

Category		(Strain / matrix) pair	Protocol	Level of detection at 50% (CFU / sample size)	
				Reference method	Alternative method
1	**Infant cereals, infant formula with or without probiotics and ingredients**	Infant formula with probiotics / *Enterobacter Kobei* Ad706	**Protocol ①**	1.50 [0.86; 2.62]	1.13 [0.66; 1.93]
			Protocol ②	1.50 [0.86; 2.62]	1.00 [0.59; 1.71]

3.2.5 Conclusion

The RLOD values (using the confirmed alternative method results) meet the acceptability limit of 2.5 for unpaired studies for the category tested and for both protocols.

The LOD values are 1.50 CFU/test portion for the reference method and 1.13 (protocol ①) and 1.00 (protocol ②) CFU/ test portion for the alternative method.

3.3 Inclusivity / exclusivity

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

3.3.1 Protocols

Inclusivity

50 target strains were freshly cultured in BHI medium at 37°C. The strain listed was defined based on the new classification of the Enterobacterales order as described by Adeolu et al. as it was previously demonstrated that the reference method is capable of detection this order.
Dilutions were made in order to inoculate 10 -100 CFU/225 ml BPW with PIF supplement, incubated for 18 h at 37°C. The alternative method protocol ① was then performed (PCR and confirmation). For protocol ②, three strains were retested based on the late Cq values obtained during the first assay (Cq>25) : *Leminorella richardii*, *Serratia fonticola*, *Pectobacterium carotovorum*

Exclusivity

30 non-target strains were freshly cultured in BHI medium at 37°C. Dilutions were made in order to inoculate about 10^5 CFU/ml BPW broth. The BPW broth was incubated for 24 h at 37°C.
These strains were selected mainly from *Gammaproteobacteria* class and *Pseudomonadota* phylum but also from other gram-negative bacteria based on their capacities to contaminate human food products and industrial environment.

3.3.2 Results

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

Inclusivity

All strains showed the expected positive PCR result. Despite the growth on both confirmation agar, the coloration variate for some strains between yellow to brown on VRBG and to green on RAPID *Enterobacteriaceae*. Positive OF glucose and negative Oxidase, confirm the appurtenance to the *Enterobacterales* order.

The three strains retested with protocol ② showed positive PCR results.

- **Exclusivity**

A total of 30 strains were tested for **exclusivity**. 29 of these strains showed the expected negative result. One strain showed a positive PCR result: *Burkholderia cepacia*. Repetition of the PCR twice showed negative results.

3.3.3 Conclusion

The alternative detection method is selective and specific.

4 INTER-LABORATORY STUDY N°2

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.

4.1 Study organisation

- *Collaborators number*

Samples were sent to 10 laboratories from six different countries with a total 16 independant collaborators involved without taking into account the expert laboratory.

- *Matrix and strain used*

Powdered infant formula with probiotics was inoculated with a lyophilized *Salmonella* Anatum Ad2706 isolated from milk powder.

- *Samples*

Samples were prepared and inoculated on Tuesday March 18, 2025, as described below:

- 24 blind coded samples (50 g) for *Enterobacteriaceae* detection by the iQ-Check EB alternative method.
- 24 blind coded samples (10 g) for *Enterobacteriaceae* detection by the ISO 21528-1 (2017) reference method.
- 1 sample (labelled “Sample for lactic acid bacteria enumeration”) for mesophilic lactic acid bacteria enumeration in MRS agar (pH 6.4).

Inoculation

The targeted inoculation levels were the following:

- Level 0: 0 CFU/test portion,
- Level 1: 1 CFU/test portion, inoculation level providing as much as possible fractional positive recovery data;
- Level 2: 2 CFU/test portion.

Each level was inoculated in bulk and thoroughly mixed prior to repartition in sterile flasks. The samples were then stored at room temperature for two weeks prior to testing.

The contamination level was determined by enumeration of the inoculum the day of the analysis in VRBG and by MPN enumeration of the inoculated bulk using ISO 21528-1 as described in the table below.

Matrix	Inoculum level	Large test Portion size	Medium test Portion size	Low test Portion size
Infant formula with probiotics	Low	5 x 20 g	8 x 10 g	5 x 5 g
	High	8 x 10 g	5 x 5 g	5 x 2 g

Labelling and shipping

Blind coded samples were placed in boxes and shipped to the different laboratories.

A temperature control flask as well as a sensor was added to the package in order to register the temperature profile during the transport, the package delivery and storage until analyses. The temperature conditions had to stay between 18°C to 27°C (ambient temperature) during transport, and during the storage.

- *Analyses*

Collaborative study laboratories and the expert laboratory carried out the analyses on April 1, 2025. The analysis by the reference method and the alternative method were performed on the same day.

The enrichment procedure chosen for the reference method was BPW + vancomycin 10mg/L + Tween 80. Oxidase and OF tests were used to confirm the colonies for both the alternative and the reference method.

For the alternative method, the protocol ① was followed by the collaborator.
The two thermocyclers (CFX96 DW System and the CFX Opus DW) were used during the study depending on the collaborator. Information is given on Table 21.

4.2 Experimental parameters controls

4.2.1 Strain stability and background microflora stability

Strain stability was checked by evaluating the bulk inoculated at the low level at different time points. Five test portions per method (reference and alternative) were tested on the day of inoculation, after 7 days and 14 days storage at ambient temperature. Lactic acid bacteria enumeration was also carried out; the results are given in Table 19.

Table 19 - Sample stability

Date of analysis	ISO 21528-1	iQ-Check EB	Lactic flora enumeration
	Nb of positives	Nb of positives	CFU/g
D+0	3/5	4/5	5.6×10^6
D+7	4/5	3/5	7.2×10^6
D+14	1/5	4/5	6.8×10^6

No evolution of the lactic flora was observed during storage at 18 – 27°C for 2 weeks. Regarding the inoculum, despite a decrease in the reference method positive results after 14 days, the alternative method showed consistent results overtime and the number of positive remained in the fractional recovery range.

4.2.2 Contamination levels

The contamination levels and the sample codification were the following (see Table 20). The contamination was determined by MPN and enumeration of the inoculum (lyophilized strain) the day of the analysis.

Table 20 - Contamination levels

Level	Samples	Theoretical target level (CFU/ test portion)	MPN (CFU/test portion)	VRBG enumeration (CFU/test portion)
0	2-5-6-9-14-19-21-24	0	0	0
	25-28-30-35-38-41-44-47			
1	3-8-11-12-15-17-20-22	1	0.79 [0.4; 1.5]	0,3
	27-32-33-37-39-42-46-48			
2	1-4-7-10-13-16-18-23	2	1.8 [0.94; 3.5]	0,6
	26-29-31-34-36-40-43-45			

The observed inoculation levels comply with the ISO 16140-2 requirement.

4.2.3 Logistic conditions

The temperatures measured at reception by the collaborators, the temperatures registered by the thermo-probe, and the receipt dates are given in Table 21.

Table 21 - Sample temperatures at receipt, date of analysis and thermocyclers used

Collaborator	Temperature measured by the probe (°C)			Temperature measured at receipt (°C)	Receipt date and time		Analysis date	Thermo-cycler
	Avg	Minimum	Maximum					
A1	20.3	12.8	22.3	17.7	27/03/2025	12h00	01/04/2025	CFX96
A2	20.3	12.5	22.5	17.8	27/03/2025	12h00	01/04/2025	CFX96
B1	18.3	12.5	22.2	10.0	27/03/2025	12h40	01/04/2025	CFX96
B2	18.3	13.0	22.1	10.0	27/03/2025	12h40	01/04/2025	CFX96
C1	18.4	13.4	21.1	20.8	27/03/2025	11h50	01/04/2025	CFX Opus
C2	18.5	12.2	22.6	21.0	27/03/2025	11h50	01/04/2025	CFX Opus
D	18.7	13.5	22.8	17.2	27/03/2025	15h00	01/04/2025	CFX96
E	20.2	12.8	22.3	13.2	27/03/2025	10h45	01/04/2025	CFX96
F1	/	/	/	/	27/03/2025	10h00	01/04/2025	CFX96

Collaborator	Temperature measured by the probe (°C)			Temperature measured at receipt (°C)	Receipt date and time		Analysis date	Thermo-cycler
	Avg	Minimum	Maximum					
F2	/	/	/	/	27/03/2025	10h00	01/04/2025	CFX96
G1	19.8	13.9	22.3	16.4	27/03/2025	11h00	01/04/2025	CFX96
G2	19.8	14.0	21.2	16.4	27/03/2025	11h00	01/04/2025	CFX96
H1	21.4	12.9	24.7	12.9	27/03/2025	11h30	01/04/2025	CFX96
H2	21.4	12.5	24.4	13.6	27/03/2025	11h30	01/04/2025	CFX96
I	18.9	10.6	21.9	11.2	27/03/2025	9h30	01/04/2025	CFX96
J	18.2	13.7	21.4	18.5	27/03/2025	10h00	01/04/2025	CFX Opus

The samples were delivered on time for all the collaborators and in good condition. Despite several demands, it was impossible to receive the temperature curves registered by the sensor for laboratory F. However, due to the low risk of failures for room temperature products and the reception at the same date as the other collaborators, it is expected that good conditions were maintained.

As the analyses were performed on time for all collaborators, they were all retained for further analysis.

4.3 Calculation and summary of data

The raw data are given in **Appendix 8**.

4.3.1 MicroVal Expert laboratory results

The results obtained by the expert laboratory are given in Table 22.

Table 22 – Results obtained by the expert Lab.

Level	Reference method	Alternative method	
		Before confirmation	After confirmation
L0	0+/8	0+/8	0+/8
L1	4+/8	7+/8	7+/8
L2	7+/8	8+/8	8+/8

Fractional recovery is fulfilled for the reference method at level 1, confirming the study was properly managed to comply with the ISO 16140-2 requirements.

Differences in the number of positives can be observed at level 1 between the reference and the alternative with consistent results between the PCR and the confirmation. The difference might be due to a normal variability of the fractional recovery.

4.3.2 Results observed by the collaborative laboratories

Lactic flora enumeration

Table 23 – Lactic flora enumeration

Collaborator	Lactic flora (CFU/g)	Analysis date
A1	<10	01/04/2025
A2	<10	01/04/2025
B1	<10	01/04/2025
B2	<10	01/04/2025
C1	<10	01/04/2025
C2	<10	01/04/2025
D	<10	01/04/2025
E	8.2×10^6	01/04/2025
F1	30	01/04/2025
F2	30	01/04/2025
G1	1.9×10^6	01/04/2025
G2	2.2×10^6	01/04/2025
H1	1.6×10^5	01/04/2025
H2	1.19×10^5	01/04/2025
I	1.1×10^6	01/04/2025
J	4.6×10^5	01/04/2025
K (ADRIA)	6.8×10^6	01/04/2025

Some collaborators obtained a result <10 CFU/g for lactic flora enumeration despite the same media has been used by all collaborators. However, results are consistent among the laboratories E, G, H, I, J and ADRIA, indicating a level between 1.6×10^5 and 8.2×10^6 CFU/g .

Enterobacteriaceae detection

16 collaborators participated in the study without considering the expert laboratory. The results obtained by the individual collaborators in the inter-laboratory are summarized in Table 24 (reference method) and Table 25 (alternative method).

Table 24 - Positive results by the reference method (ALL the collaborators except ADRIA)

Collaborators	Contamination level		
	L0	L1	L2
A1	1	7	7
A2	0	5	5
B1	1	7	6
B2	1	4	6
C1	0	4	6
C2	0	4	6
D	0	6	8
E	0	8	7
F1	0	4	6
F2	0	4	6
G1	0	4	8
G2	0	5	3
H1	0	7	5
H2	0	6	7
I	unk	unk	6
J	0	6	6
K (ADRIA)	0	4	7
Total	$P_0 = 3$	$P_1 = 85$	$P_2 = 105$

unk: unknown results due to the mixing of two samples between the blank and low contamination level

Table 25 - 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
A1	0	0	0	4	4	4	7	7	7
A2	1	0	0	5	5	5	7	7	7
B1	1	1	1	6	6	6	6	6	6
B2	1	2	1	4	5	4	7	7	7
C1	0	0	0	5	6	5	8	8	8
C2	0	0	0	6	6	6	7	7	7
D	0	0	0	7	7	7	8	8	8
E	0	0	0	4	4	4	7	7	7
F1	0	0	0	7	7	7	7	7	7
F2	0	0	0	7	7	7	8	8	8
G1	0	0	0	7	7	7	8	8	8
G2	0	0	0	2	2	2	7	7	7
H1	1	0	0	5	5	5	8	8	8
H2	0	0	0	6	6	6	6	6	6
I	3	0	0	6	6	6	7	7	7
J	3	0	0	5	5	5	4	7	4
K (ADRIA)	0	0	0	7	7	7	8	8	8
Total	**P_0 =10**	**C_0 = 3**	**CP_0 = 2**	**P_1 = 93**	**C_1 = 95**	**CP_1 = 93**	**P_2 = 120**	**C_2 = 123**	**CP_2 = 120**

During the analysis, three collaborators didn't follow correctly the process : A1 and A2 performed the confirmation for the PCR negative samples only after 5 days of storage and for collaborator I, a mix of two samples (one blank and one low level) was done. To ensure the reliability of the analysis, it was decided to remove these collaborators from the analysis.

For collaborators B1 and B2, cross-contaminations were observed with both reference and alternative methods on at least one blank sample per method. The contamination was confirmed on plates, indicating a probable issue with handling practices. As it is therefore not possible to determine the impact on other contamination levels, it is proposed to exclude these collaborators from the analysis.

Finally, for collaborators H1 and J, false positive results at the blank level with the alternative method can be observed (1 for H1 and 3 for J). The contamination was not

confirmed on the plates indicating an issue during the extraction or the PCR only. Repetition of the PCR test showed negative results most of the time, except for one sample for collaborator J (n°24).
Interestingly, for collaborator J, false negative results are also obtained at level 2. Given the number of discrepancies observed in this laboratory (3 $PD_{FP(alt)}$ at level 0 and 3 $ND_{FN(alt)}$ at level 2) there is a strong indication of sample mix-up during the PCR step. This suspicion is reinforced by the close numbering of the samples, suggesting they may have been handled together. Considering these deviations, it was agreed with the technical committee to remove this laboratory from the analysis.

Therefore, 10 datasets are available for interpretation without considering the expert laboratory dataset.

4.3.3 Results of the collaborators retained for interpretation

The results obtained with the 11 collaborators kept for interpretation are presented in Table 26 (reference method) and Table 27 (alternative method).

Table 26 - Positive results by the reference method
(Without Labs A1, A2, B1, B2, I and J)

Collaborators	Contamination level		
	L0	L1	L2
C1	0	4	6
C2	0	4	6
D	0	6	8
E	0	8	7
F1	0	4	6
F2	0	4	6
G1	0	4	8
G2	0	5	3
H1	0	7	5
H2	0	6	7
TOTAL	$P_0 = 0$	$P_1 = 52$	$P_2 = 62$

Table 27 - Positive results (before and after confirmation) by the alternative method (Without Labs A1, A2, B1, B2, and I)

Collaborators	Contamination level								
	L0			*L1*			*L2*		
	PCR result	Confirmation result	Final result	PCR result	Confirmation result	Final result	PCR result	Confirmation result	Final result
C1	0	0	0	5	6	5	8	8	8
C2	0	0	0	6	6	6	7	7	7
D	0	0	0	7	7	7	8	8	8
E	0	0	0	4	4	4	6	7	7
F1	0	0	0	7	7	7	7	7	7
F2	0	0	0	7	7	7	8	8	8
G1	0	0	0	7	7	7	8	8	8
G2	0	0	0	2	2	2	7	7	7
H1	1	0	0	5	5	5	8	8	8
H2	0	0	0	6	6	6	6	6	6
TOTAL	**P_0 =1**	**C_0 = 0**	**CP_0 = 0**	**P_1 = 56**	**C_1 = 57**	**CP_1 = 56**	**P_2 = 73**	**C_2 = 74**	**CP_2 = 74**

4.3.4 Calculation of the specificity percentage (SP)

As described in paragraph 3.3.2, one false positive PCR results were obtained with the alternative method, all at level 0. After repetition of the PCR twice, no positive was found, suggesting possible cross contamination or interference during the initial test. No false positive was observed by the expert lab or by the other 9 collaborators retained for interpretation.

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 28).

Table 28 - Percentage specificity (Without Labs A1, A2, B1, B2, and I)

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

4.3.5 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 both inoculation level (L1 and L2). These inoculation levels were retained for calculation.

A summary of the results of the collaborators retained for interpretation and obtained with reference and the alternative methods for Level 1 are provided in Table 29.

Table 29 - Summary of the results obtained with the reference method and the alternative method for Level 1 (Without Labs A1, A2, B1, B2, I and J)

Level	Response	Reference method positive (R+)	Reference method negative (R-)
1	Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 35**	Positive deviation (R-/A+) **PD = 21**
	Alternative method negative (A-)	Negative deviation (A-/R+) **TND = 17 (with 0 $ND_{FN(alt)}$ and 0 $PA_{FP(alt)}$)**	Negative agreement (A-/R-) **TNA = 7 (with 1 $NA_{FN(alt)}$ and 0 $PD_{FP(alt)}$))**
2	Alternative method positive (A+)	Positive agreement (A+/R+) **PA = 57**	Positive deviation (R-/A+) **PD = 16**
	Alternative method negative (A-)	Negative deviation (A-/R+) **TND = 5 (with 1 $ND_{FN(alt)}$ and 0 $PA_{FP(alt)}$)**	Negative agreement (A-/R-) **TNA = 2 (with 0 $NA_{FN(alt)}$ and 0 $PD_{FP(alt)}$))**

With $TNA = NA + NA_{FN\,(alt)} + PD_{FP\,(alt)}$ $TND = ND + ND_{FN\,(alt)} + PA_{FP\,(alt)}$

Based on the data summarized in Table 29, 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 30).

Table 30 - Sensitivity, relative trueness and false positive ratio percentages

		Level 1	Level 2
Sensitivity for the alternative method:	$SE_{alt} = \frac{(PA + PD)}{(PA + TND + PD)} \; x \; 100\,\%$	76.7 %	93.6 %
Sensitivity for the reference method:	$SE_{ref} = \frac{(PA + TND)}{(PA + TND + PD)} \; x \; 100\,\%$	71.2 %	79.5 %
Relative trueness	$RT = \frac{(PA + TNA)}{N} x 100\,\%$	52.5 %	73.8 %
False positive ratio for the alternative method (unpaired evaluation)	$FPR = \frac{PA_{FP(alt)} + PD_{FP\,(alt)}}{TNA} \; x \; 100\,\%$	0.0 %	0.0 %
False negative ratio for the alternative method (unpaired evaluation)	$FNR = \frac{NA_{FN(alt)} + ND_{FN(alt)}}{PA + TND + PD}$	0.014	0.013

4.3.6 Interpretation of data

Negative deviations are listed in Table 31 for Level 1 and in Table 32 for Level 2. Positive deviations are listed in Table 33 for Level 1 and in Table 34 for Level 2.

Table 31 – Total negative deviations for Level 1

Lab	Sample N°	iQ-Check *Enterobacteriaceae*		Confirmation			Agreement	Thermocycler
		Target Cq (FAM)	IC Cq (HEX)	RAPID' *EB*	VRBG	Colony confirmation		
C1	12	N/A	30.62	-	-	/	ND	CFX Opus
C2	32	N/A	30.62	-	-	/	ND	CFX Opus
C2	46	N/A	30.75	-	-	/	ND	CFX Opus
D	20	N/A	30.43	-	-	/	ND	CFX96
E	3	N/A	30.69	-	-	/	ND	CFX96
E	8	N/A	31.63	-	-	/	ND	CFX96
E	12	N/A	30.76	-	-	/	ND	CFX96
E	22	N/A	30.95	-	-	/	ND	CFX96
G1	20	N/A	30.74	-	-	/	ND	CFX96
G2	27	N/A	32.12	-	-	/	ND	CFX96
G2	33	N/A	30.33	-	-	/	ND	CFX96
G2	37	N/A	30.63	-	-	/	ND	CFX96
G2	46	N/A	30.84	-	-	/	ND	CFX96
H1	8	N/A	30.7	-	-	/	ND	CFX96
H1	11	N/A	30.62	-	-	/	ND	CFX96
H1	22	N/A	30.46	-	-	/	ND	CFX96

Lab	Sample N°	iQ-Check *Enterobacteriaceae*		Confirmation			Agreement	Thermocycler
		Target Cq (FAM)	IC Cq (HEX)	RAPID' *EB*	VRBG	Colony confirmation		
H2	**32**	N/A	30.76	-	-	/	**ND**	**CFX96**

Table 32 – Total negative deviations for Level 2

Lab	Sample N°	iQ-Check *Enterobacteriaceae*		Confirmation			Agreement	Thermocycler
		Target Cq (FAM)	IC Cq (HEX)	RAPID' *EB*	VRBG	Colony confirmation		
C2	**31**	N/A	30.82	-	-	/	**ND**	**CFX Opus**
E	**10**	N/A	30,76	-	-	/	**ND**	**CFX96**
E	**16**	N/A 35.69/ 34.19	31.51/ 30.77/ 30.65	+	+	+	**$ND_{FN(alt)}$**	**CFX96**
G2	**29**	N/A	30.7	-	-	/	**ND**	**CFX96**
H2	**26**	N/A	30.68	-	-	/	**ND**	**CFX96**

At level 1, consistent results were obtained between PCR and confirmation for the negative deviations.

At level 2, one $N_{FN(alt)}$ result (Lab E) was observed among the 5 TND samples. The repetition of the PCR showed late positive curves indicating a growth probably at the limit of detection of the alternative method. No picture of the plate was available to determine the level of the contamination.
Additionally, one sample was found $NA_{FN(alt)}$ for laboratory C1 (at the low level: sample n°15). Repetition of the PCR did not allow to recover a positive PCR result.
It was not possible to obtain the number of colonies on confirmation plate to confirm the growth level at the end of the enrichment.

Table 33 – Positive deviations for Level 1

Lab	Sample N°	iQ-Check *Enterobacteriaceae*		Confirmation			Agreement	Thermocycler
		Target Cq (FAM)	IC Cq (HEX)	RAPID' *EB*	VRBG	Colony confirmation		
C1	11	31.28	30.37	+	+	+	PD	CFX Opus
C1	20	27.51	30.14	+	+	+	PD	CFX Opus
C2	33	25.98	30.17	+	+	+	PD	CFX Opus
C2	37	18.63	30.64	+	+	+	PD	CFX Opus
C2	39	18.67	31.08	+	+	+	PD	CFX Opus
C2	48	19.41	31.49	+	+	+	PD	CFX Opus
D	15	18.14	30.98	+	+	+	PD	CFX96
D	17	20.04	31.14	+	+	+	PD	CFX96
F1	3	18.02	31.99	+	+	+	PD	CFX96
F1	8	18.41	32.04	+	+	+	PD	CFX96
F1	12	18.26	31.77	+	+	+	PD	CFX96
F2	39	18.25	32.38	+	+	+	PD	CFX96
F2	46	17.81	32.9	+	+	+	PD	CFX96
F2	48	17.83	31.95	+	+	+	PD	CFX96
G1	8	18.87	30.87	+	+	+	PD	CFX96
G1	11	18.85	36.24	+	+	+	PD	CFX96
G1	17	18.64	35.23	+	+	+	PD	CFX96
G1	22	22.33	30.51	+	+	+	PD	CFX96
G2	48	21.86	30.15	+	+	+	PD	CFX96
H1	15	25.99	30.25	+	+	+	PD	CFX96
H2	33	26.36	30.34	+	+	+	PD	CFX96

Table 34 – Positive deviations for Level 2

Lab	Sample N°	iQ-Check *Enterobacteriaceae*		Confirmation			Agreement	Thermocycler
		Target Cq (FAM)	IC Cq (HEX)	RAPID' *EB*	VRBG	Colony confirmation		
C1	4	30.61	30.34	+	+	+	PD	CFX Opus
C1	13	18.34	30.66	+	+	+	PD	CFX Opus
C2	29	18.63	30.78	+	+	+	PD	CFX Opus
C2	36	18.63	31.31	+	+	+	PD	CFX Opus
E	4	29,09	30,68	+	+	+	PD	CFX96
G2	26	19.04	31.84	+	+	+	PD	CFX96
G2	31	18.03	32.4	+	+	+	PD	CFX96
G2	34	17.75	32.48	+	+	+	PD	CFX96
G2	40	22.26	30.25	+	+	+	PD	CFX96
G2	43	18.46	30.72	+	+	+	PD	CFX96
H1	1	17.58	32.45	+	+	+	PD	CFX96
H1	4	18.43	31.39	+	+	+	PD	CFX96
H1	10	22.5	30.3	+	+	+	PD	CFX96

The positive deviations showed consistent result between PCR and both confirmations plates. They are not associated with any specific laboratory, or thermocycler.

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

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

where

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

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

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

where

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

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

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

where

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

The AL is not met when the observed value (TND - PD) 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 reason 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 and Level 2. The calculations, according to the ISO 16140-2 are shown in table 35.

Table 35 – Calculations (Without Labs A1, A2, B1, B2, I and J)

	Level 1	Level 2
N_X	80	80
$(p+)_{ref}$	0.65	0.78
$(p+)_{alt}$	0.70	0.92
AL = (TND - PD) max	10.28	8.10
TND - PD	-4	-11
Conclusion	PASS	PASS

The ISO 16140-2 and ISO 16140-2/A1 requirements are fulfilled as (TND - PD) meet the AL for both inoculation levels.

4.3.7 Evaluation of the LOD_{50} and RLOD between laboratories

The $LOD_{50\%}$ was calculated using the ISO 16140-2 Excel spreadsheet available at https://standards.iso.org/iso/16140/-2/ed-1/en/amd/1/PODLOD-interlab_ver2.xlsm.

The RLOD is defined as the ratio of the LODs of the alternative method and the reference method: **$RLOD = LOD_{alt}/LOD_{ref}$**. The results are given in Table 36.

Note that the calculation did not consider laboratories A and D2 as positive results were obtained at level 0 for those participants.

Table 36 - $LOD_{50\%}$ and RLOD (Without Labs A1, A2, B1, B2, I and J)

Method	LOD 50%	RLOD
Reference	0.7 [0.5; 0.8]	0.8
Alternative	0.5 [0.4; 0.6]	

5 GENERAL CONCLUSION

The **method comparison study conclusions** are:

- The observed values for TND-PD for the individual categories and for all categories meet the acceptability limits (observed values ≤ AL).

- The RLOD values (using the confirmed alternative method results) meet the acceptability limit, of 2.5 for unpaired studies for the category tested.

- The alternative iQ-Check EB detection method is selective and specific.

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

- The interlaboratory study n°2 was performed as an unpaired study design on 50 g Infant formula with probiotics inoculated with a lyophilized strain *(S. anatum)*.

- 16 collaborators from 10 laboratories were recruited for the study; 11or 10 were retained for interpretation.

- Testing was performed in good conditions and both inoculation levels were found in fractional recovery.

- The TND-PD results meet the AL calculated at both inoculation level.

These data and interpretations comply with the ISO 16140-2, ISO 16140-2/A1 and MicroVal technical rules. requirements.

The iQ-Check *Enterobacteriaceae* kit is considered equivalent to the ISO 21528-1 standard method for *Enterobacteriaceae* detection.

Quimper, 02 September 2025

Florian QUERO	Astrid CARIOU
Technical Study Manager	Manager
Method performance in food microbiology	Method performance in food microbiology
I hereby attest to the validation of the results of the analyses carried out under the COFRAC accreditation.	I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).

6 REFERENCES

- ISO 4833-1:2013; Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms -- Part 1: Colony count at 30 ° C by the pour plate technique

- ISO 15214: 1998; Microbiology of the food chain -- Horizontal method for the enumeration of mesophilic lactic acid bacteria -Colony count at 30 °C

- ISO 21528-1:2017: Microbiology of the food chain – Horizontal method for the detection of *Enterobacteriaceae* – Part 1: detection of *Enterobacteriaceae*

- ISO 6887; Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Parts 1-4 and 5.

- ISO 7218; Microbiology of food and animal feeding stuffs -- General requirements and guidance for microbiological examinations.

- ISO 16140-2:2016/Amd 1:2024; Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. Amendment 1: Revision of qualitative method comparison study data evaluation, relative level of detection calculations in the interlaboratory study, calculation and interpretation of the relative trueness study, and inclusion of a commercial sterility testing protocol for specific products

- 2016-028 (GC) Proposed MicroVal Technical committee interpretation of ISO 16140-2 and ISO

- 2022-069 (TC) MVTC interpretation of ISO 16140-2-6 V2-5

- Adeolu M, Alnajar S, Naushad S, S Gupta R. Genome-based phylogeny and taxonomy of the *'Enterobacteriales':* proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae, Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov.; Int J Syst Evol Microbiol. 2016 Dec;66(12):5575-5599.

Appendix 1 - Flow diagram of the reference method: ISO 21528-1:2017: Microbiology of the food chain – Horizontal method for the detection of *Enterobacteriaceae* – Part 1: detection of *Enterobacteriaceae*

10 g of sample + 90 ml BPW + vancomycin (10mg/L) + Tween 80 (10g/L)

↓

18 h ± 2 h at 37°C ± 1°C

↓

Streak 10 µl onto VRBG

Incubation 24 h ± 2 h at 37°C ± 1°C

Test one characteristic colony (pink to purple)
(Take 4 other colonies if the first one is negative)

Streak onto non-selective agar plate
Incubation for 24 h ± 2 h at 37°C ± 1°C

↓

Oxidase test and Glucose fermentation in OF media (24h ± 2h at 37°C ± 1°C): *Enterobacteriaceae*: Oxidase negative, Glucose fermentation positive (OF: yellow color)

Appendix 2 - Flow diagram of the alternative method: iQ-Check EB

375 g* + 1125 ml prewarmed BPW + PIF supplement
with addition of alpha amylase for cereals
↓
Incubation 18 - 26 h at 37 ± 1°C
Possibility to store the enriched samples for 72 h at 5 ±3°C

FDRS protocol

Rehydrate Reagent G1 in 1 ml distilled water for 5 - 10 min at room temperature
↓
Transfer G1 to the activation buffer (G2). Mix by inverting the tube
↓
Pipette 10 µl of activate reagent into the bottom of each well of a 96-Deep Well microplate
↓
Add 100 µl of decanted enriched broth per well
↓
Seal the Deep Well microplate with the X-Pierce sealing film
↓
Incubate in the thermoshaker without shaking for 15 to 30 min at 37°C

Easy I protocol:

Add 100 µl lysis reagent in a tube or a Deep Well plate
↓
Add 100 µL of FDRS treated sample
↓
Agitation for 3 min ± 1 min with the cell disruptor (for tubes only)
↓
Incubation at 95 - 100°C for 15 - 20 min in a heat block (tubes) or in the plate agitator-incubator (plates and tubes) under agitation at 1300 rpm

Centrifugation for 5 min at 10 000 g (tubes only)
Cool down to room temperature if using a deep well plate

Transfer 5 µl of supernatant in a PCR tube containing 45 µl of mix PCR reagents
↓
PCR using the CFX96 Deep Well or the CFX Opus Deepwell thermocycler (APF Fast)
↓
Confirmation of positive PCR results
↓
Reference method protocol:
10 µl of the primary enriched broth onto VRBG
Incubation for 24 ± 2 h at 37 ± 1°C
Colony confirmation using Oxidase and OF test

Alternative method protocol:
10 µl of the primary enriched broth onto VRBG or RAPID *Enterobacteriaceae*
Incubation 24 ± 2 h at 37 ± 1°C
The only presence of typical colonies allows to confirm the positive PCR results

* 50g test portion was used for the ILS study

Appendix 3 - Kit insert(s)

See attached

Appendix 4 - Artificial inoculations

Year of analysis	Sample N°	Product (French name)	Product	Artificial contamination				ISO method Enrichment broth	Global results Protocol ①	Global result Protocol ②	Category	Type
				Strain	Origin	Injury protocol	Inoculation level CFU/sample					
2024	**1432**	**Poudre de lait infantile dès 6 mois (37% MG)**	**Infant formula (37% fat level)**	*Klebsiella pneumoniae* 120	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW + Tween 80	-	-	1	a
2024	**1433**	**Poudre de lait infantile 6-12 mois bio (23% MG)**	**Infant formula (23% fat level)**	*Klebsiella pneumoniae* 120	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW + Tween 80	+	+	1	a
2024	**1434**	**Poudre de lait infantile 6-12 mois (32% MG)**	**Infant formula (32% fat level)**	*Klebsiella pneumoniae* 120	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW + Tween 80	+	+	1	a
2024	**1435**	**Poudre de lait système immunitaire 6-12 mois (22% MG)**	**Infant formula immune system (22% fat level)**	*Serratia macescens* Ad455	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW + Tween 80	-	-	1	a
2024	**1436**	**Poudre de lait infantile dès 6 mois (23.6% MG)**	**Infant formula (23.6% fat level)**	*Serratia macescens* Ad455	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW + Tween 80	-	+	1	a
2024	**1437**	**Céréales infantiles saveur biscuit**	**Infant cereals, bicscuit taste**	*Cronobacter dublinensis* Ad2848	Infant cereals	Seeding lyophilised strain 2 weeks at ambient temperature	0.8	BPW + α-amylase	+	+	1	a
2024	**1438**	**Céréales infantiles légumes du potager**	**Infant cereals, vegetables**	*Cronobacter dublinensis* Ad2848	Infant cereals	Seeding lyophilised strain 2 weeks at ambient temperature	0.8	BPW + α-amylase	+	+	1	a
2024	**1439**	**Céréales infantiles multicéréales/légumes du soleil dès 8 mois**	**Infant cereals, multicereals, vegetables**	*Cronobacter dublinensis* Ad2848	Infant cereals	Seeding lyophilised strain 2 weeks at ambient temperature	0.8	BPW + α-amylase	+	+	1	a
2024	**1440**	**Céréales infantiles 5 céréales/miel 8-36 mois**	**Infant cereals, vegetables, honey**	*Klebsiella pneumoniae* 120	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW + α-amylase	-	-	1	a
2024	**1441**	**Céréales infantiles 3 céréales**	**Infant cereals**	*Klebsiella pneumoniae* 120	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW + α-amylase	+	+	1	a
2024	**1442**	**Maltodextrine**	**Maltodextrin**	*Escherichia hermanii* Ad464	Raw milk	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	+	+	1	c
2024	**1443**	**Lactose**	**Lactose**	*Escherichia hermanii* Ad464	Raw milk	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	+	+	1	c
2024	**1444**	**Protéine de lactoserum**	**Whey proteins**	*Escherichia hermanii* Ad464	Raw milk	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	+	+	1	c
2024	**1445**	**Caséinate**	**Caseinate**	*Serratia macescens* Ad455	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	-	-	1	c
2024	**1446**	**Amidon de blé**	**Wheat starch**	*Serratia macescens* Ad455	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	+	+	1	c
2024	**1448**	**Poudre de lait infantile 6 mois - 1 an avec probiotiques (*S. thermophilus*: 1.7 x 10^4 UFC/g) (24.3% MG)**	**Infant formula with probiotics (*S. thermophilus*: 1.7 x 10^4 CFU/g) (24.3% fat level)**	*Klebsiella pneumoniae* 120	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW 2X + Tween 80	+	+	1	b
2024	**1449**	**Poudre de lait infantile 0-6 mois avec probiotiques (*B. lactis*: 5.3 x 10^3 UFC/g) (28.2% MG)**	**Infant formula with probiotics (*B. lactis*: 5.3 x 10^3 CFU/g) (28.2% fat level)**	*Escherichia hermanii* Ad464	Raw milk	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW 2X + Tween 80	+	+	1	b
2024	**1450**	**Poudre de lait infantiles dès 6 mois avec probiotiques (*L. imosilactobabillus*: 1.1 x 10^5 UFC/g) (>20% MG)**	**Infant formula with probiotics (*L. imosilactobabillus*: 1.1 x 10^5 CFU/g) (>20% fat level)**	*Escherichia hermanii* Ad464	Raw milk	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW 2X + Tween 80	+	+	1	b
2024	**1451**	**Poudre de lait infantile 6 mois - 1 an avec probiotiques (*L. reuteri*: 3.2 x 10^4 UFC/g) (24.3% MG)**	**Infant formula with probiotics (*L. reuteri*: 3.2 x 10^4 CFU/g) (24.3% fat level)**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW 2X + Tween 80	-	-	1	b

Year of analysis	Sample N°	Product (French name)	Product	Artificial contamination				ISO method Enrichment broth	Global results Protocol ①	Global result Protocol ②	Category	Type
				Strain	Origin	Injury protocol	Inoculation level CFU/sample					
2024	**1452**	**Poudre de lait infantile dès 6 mois avec probiotiques (*L. reuteri*: 3.1 x 10^5 UFC/g) (27.6% MG)**	**Infant formula with probiotics (*L. reuteri*: 3.1 x 10^5 CFU/g) (27.6% fat level)**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW 2X + Tween 80	+	+	1	b
2024	**1453**	**Céréales infantiles lentilles. carottes, pois avec probiotiques (*B. lactis*: 2.7 x 10^3 UFC/g)**	**Infant formula with probiotics (*B. lactis*: 2.7 x 10^3 CFU/g)**	*Cronobacter dublinensis* Ad2848	Infant cereals	Seeding lyophilised strain 2 weeks at ambient temperature	0.8	BPW 2X + α-amylase	+	-	1	b
2024	**1454**	**Céréales infantiles saveur banane avec probiotiques (*B. lactis*: 3.9 x 10^5 UFC/g)**	**Infant formula with probiotics (*B. lactis*: 3.9 x 10^5 CFU/g)**	*Cronobacter dublinensis* Ad2848	Infant cereals	Seeding lyophilised strain 2 weeks at ambient temperature	0.8	BPW 2X + α-amylase	+	-	1	b
2024	**1455**	**Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 2.6 x 10^4 UFC/g)**	**Infant formula with probiotics (Bifidobacteria:2.6 x 10^4 CFU/g)**	*Cronobacter dublinensis* Ad2848	Infant cereals	Seeding lyophilised strain 2 weeks at ambient temperature	0.8	BPW 2X + α-amylase	+	+	1	b
2024	**1456**	**Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 1.7 10^4 UFC/g)**	**Infant formula with probiotics (Bifidobacteria: 1.7 x 10^4 CFU/g)**	*Lelliottia amnigena* 129	Milk product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW 2X + α-amylase	+	+	1	b
2024	**1457**	**Céréales infantiles lentilles, pêche, pomme avec probiotiques (*B. lactis*: 1.1 x 10^6 UFC/g)**	**Infant formula with probiotics(*B. lactis*: 1.1 x 10^6 CFU/g)**	*Lelliottia amnigena* 129	Milk product	Seeding lyophilised strain 2 weeks at ambient temperature	1	BPW 2X + α-amylase	+	+	1	b
2024	**1458**	**Lactose**	**Lactose**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	+	+	1	c
2024	**1459**	**Lactoserum**	**Whey**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	+	+	1	c
2024	**1460**	**Amidon de mais**	**Corn starch**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	<1	BPW	-	-	1	c
2024	**1461**	**Amidon de riz**	**Rice starch**	*Salmonella* Anatum Ad2718	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	5	BPW	+	+	1	c
2024	**1462**	**Amidon de blé**	**Wheat starch**	*Salmonella* Anatum Ad2718	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	5	BPW	+	+	1	c
2024	**1910**	**Poudre de lait infantile 0-6 mois (>20% MG)**	**Infant formula**	*Salmonella* Anatum Ad2718	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	0.5	BPW + Tween 80	+	+	1	a
2024	**1911**	**Poudre de lait infantile 6-125 mois (22% MG)**	**Infant formula**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1.2	BPW + Tween 80	+	+	1	a
2024	**1912**	**Poudre de lait infantile 6-12 mois (26% MG)**	**Infant formula**	*Hafnia alvei* Ad2274	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	1.2	BPW + Tween 80	+	+	1	a
2024	**1913**	**Céréales infantiles 6-36 mois**	**Infant cereals**	*Lelliottia amnigena* 129	Milk product	Seeding lyophilised strain 2 weeks at ambient temperature	3	BPW + α-amylase	+	+	1	a
2024	**1914**	**Céréales infantiles dès 6 mois**	**Infant cereals**	*Enterobacter kobei* Ad706	Milk powder	Seeding lyophilised strain 2 weeks at ambient temperature	1.2	BPW + α-amylase	+	+	1	a
2024	**1915**	**Poudre de lait infantile 0-6 mois avec probiotiques (*L. reuteri*: 1.1 x 10^7 UFC/g) (27.8% MG)**	**Infant formula with probiotics (*L. reuteri*:1.1 x 10^7 CFU/g) (27.8% fat level)**	*Serratia macescens* Ad455	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	0.7	BPW 2X + Tween 80	+	+	1	b
2024	**1916**	**Poudre de lait infantile bio 10 mois - 3 ans avec probiotiques (*L. reuteri*:1.5 x 10^6 UFC/g) (24% MG)**	**Infant formula with probiotics (*L. reuteri*:1.5 x 10^6 CFU/g) (24% fat level)**	*Enterobacter kobei* Ad706	Milk powder	Seeding lyophilised strain 2 weeks at ambient temperature	1.2	BPW 2X + Tween 80	+	+	1	b

Year of analysis	Sample N°	Product (French name)	Product	Artificial contamination				ISO method Enrichment broth	Global results Protocol ①	Global result Protocol ②	Category	Type
				Strain	Origin	Injury protocol	Inoculation level CFU/sample					
2024	**1917**	**Poudre de lait infantile 0-6 mois avec probiotiques (Limocilactobacillus: 6.3 x 10^6 UFC/g) (>20% MG)**	**Infant formula with probiotics (*Limocilactobacillus*: 6.3 x 10^6 CFU/g) (>20% fat level)**	*Enterobacter kobei* Ad706	Milk powder	Seeding lyophilised strain 2 weeks at ambient temperature	1.2	BPW 2X + Tween 80	+	+	1	b
2024	**1918**	**Céréales infanitle pêche/pomme avec probiotiques (*B. lactis*: 1.2 x 10^6 UFC/g)**	**Infant cereals with probiotics (*B. lactis*: 1.2 x 10^6 CFU/g)**	*Salmonella* Anatum Ad2718	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	0.5	BPW 2X + Tween 80	+	+	1	b
2024	**1919**	**Maltodextrine**	**Maltodextrin**	*Serratia macescens* Ad455	Dairy product	Seeding lyophilised strain 2 weeks at ambient temperature	0.7	BPW	+	+	1	c
2024	**1920**	**Amidon de mais**	**Corn starch**	*Enterobacter kobei* Ad706	Milk powder	Seeding lyophilised strain 2 weeks at ambient temperature	1.2	BPW	-	-	1	c
2024	**1922**	**Lactose**	**Lactose**	*Lelliottia amnigena* 129	Milk product	Seeding lyophilised strain 2 weeks at ambient temperature	3	BPW	+	+	1	a
2024	**2469**	**Poudre de lait écrémé**	**Non-fat level dry milk**	*Salmonella* Goldcoast Ad3006	Dairy product	Seeding lyophilised strain 1 week at ambient temperature	2,3	BPW	+	+	1	c
2024	**2470**	**Poudre de lait écrémé**	**Non-fat level dry milk**	*Salmonella* Derby Ad3381	Dairy product	Seeding lyophilised strain 1 week at ambient temperature	0,7	BPW	+	+	1	c
2024	**2471**	**Poudre de lait écrémé**	**Non-fat level dry milk**	*Salmonella* Duisburg Ad1812	Dairy product	Seeding lyophilised strain 1 week at ambient temperature	4	BPW	+	+	1	c

Appendix 5 - Sensitivity study: raw data

INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																																
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	**Reference method: ISO 21528-1♦**				**Alternative method: iQ-Check *Enterobacteriaceae***																							
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																							
									PCR result			PCR result			Confirmation																
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																			
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG												
					Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type				
2024	**1432**	**Poudre de lait infantile dès 6 mois (37% MG)**	**Infant formula (37% fat level)**	BPW + Tween 80	st	/	/	-	33.90/ 37.65/ -	32.29/ 32.88/ 31.89	+/+/-	N/A	33.64	-	st	/	/	st	/	/	-	-	-	**$PD_{FP(alt)}$**	**NA**	1	a				
2024	**1433**	**Poudre de lait infantile 6-12 mois bio (23% MG)**	**Infant formula (23% fat level)**	BPW + Tween 80	st	/	/	-	23.98	33.25	+	24.32	N/A	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a				
2024	**1434**	**Poudre de lait infantile 6-12 mois (32% MG)**	**Infant formula (32% fat level)**	BPW + Tween 80	+p	-	+	+	21.37	32.68	+	23.49	33.69	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a				
2024	**1435**	**Poudre de lait système immunitaire 6-12 mois (22% MG)**	**Infant formula immune system (22% fat level)**	BPW + Tween 80	st	/	/	-	N/A	32.46	-	N/A	33.47	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	a				
2024	**1436**	**Poudre de lait infantile dès 6 mois (23.6% MG)**	**Infant formula (23.6% fat level)**	BPW + Tween 80	st	/	/	-	N/A/20.92/ 21.14/37.99/ 37.47/N/A/ 36.03/N/A/ 37.41/38.42	32.63/32.03/ 32.29/32.39/ 32.29/32.55/ 31.98/32.61/ 32.43/32.43	- /+/+/+/+/- /+/-/+/+	37.50	33.03	+	+p	-	+	+p	-	+	+	-	+	**$NA_{FN(alt)}$**	**PD**	1	a				
2024	**1437**	**Céréales infantiles saveur biscuit**	**Infant cereals, biscuit taste**	BPW + α-amylase	d	-	+	+	N/A	34.75	-	N/A	32.85	-	st	/	/	st	/	/	-	-	-	**ND**	**ND**	1	a				
2024	**1438**	**Céréales infantiles légumes du potager**	**Infant cereals, vegetables**	BPW + α-amylase	st	/	/	-	17.76	N/A	+	20.53	36.88	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a				
2024	**1439**	**Céréales infantiles multicéréales/légumes du soleil dès 8 mois**	**Infant cereals, multicereals, vegetables**	BPW + α-amylase	st	/	/	-	37.31	36.46	+	28.23	32.64	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a				
2024	**1440**	**Céréales infantiles 5 céréales/miel 8-36 mois**	**Infant cereals, vegetables, honey**	BPW + α-amylase	st	/	/	-	N/A	34.02	-	N/A	33.41	-	st	/	/	-C	/	/	-	-	-	**NA**	**NA**	1	a				

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

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INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																														
					Reference method: ISO 21528-1♦				**Alternative method: iQ-Check *Enterobacteriaceae***																					
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																				
									PCR result			PCR result			Confirmation														
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																	
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG										
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type
2024	**1441**	**Céréales infantiles 3 céréales**	**Infant cereals**	BPW + α-amylase	+p	-	+	+	34.94	33.73	+	27.65	32.16	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1910**	**Poudre de lait infantile 0-6 mois (>20% MG)**	**Infant formula**	BPW + Tween 80	+m	-	+	+	28.57	N/A	+	20.55	N/A	+	+M	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1911**	**Poudre de lait infantile 6-125 mois (22% MG)**	**Infant formula**	BPW + Tween 80	+M	-	+	+	23.17	31.65	+	26.37	31.74	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1912**	**Poudre de lait infantile 6-12 mois (26% MG)**	**Infant formula**	BPW + Tween 80	+M	-	+	+	27.69	31.22	+	27.24	31.08	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1913**	**Céréales infantiles 6-36 mois**	**Infant cereals**	BPW + α-amylase	+p	-	+	+	36.33	32.16	+	N/A/N/ A/N/A	32.21/ 34.14/ 34.29	-/-/-	+p	-	+	+p	-	+	+	+	-	**PA**	**$ND_{FN(alt)}$**	1	a
2024	**1914**	**Céréales infantiles dès 6 mois**	**Infant cereals**	BPW + α-amylase	+p	-	+	+	N/A	32.59	-	N/A	32.55	-	st	/	/	st	/	/	-	-	-	**ND**	**ND**	1	a
2024	**1922**	**Lactose**	**Lactose**	BPW	+p	-	+	+	37.50	32.45	+	36.93	32.46	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**2227**	Poudre de lait infantile bio 6-12 mois (23% MG)	Infant formula (23% fat level)	BPW + Tween 80	st	/	/	-	N/A	32.27	-	N/A	33.02	-	-C	/	/	-C	/	/	-	-	-	**NA**	**NA**	1	a
2024	**2228**	Poudre de lait infantile 6-12 mois (23.6% MG)	Infant formula (23.6% fat level)	BPW + Tween 80	st	/	/	-	N/A	32.32	-	N/A	32.97	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	a
2024	**2229**	Poudre de lait infantile 6-12 mois (22.6% MG)	Infant formula (22.6% fat level)	BPW + Tween 80	st	/	/	-	N/A	33.43	-	N/A	32.23	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	a
2024	**2230**	Céréales infantiles saveur biscuit	Infant cereals, biscuit taste	BPW + α-amylase	d	- x5	+ x5	+	30.23	32.93	+	29.64	31.52	+	-A	/	/	-A	/	/	- (+ at 72h: Pantoea spp))	+	+	**PA**	**PA**	1	a
2024	**2231**	Céréales infantiles saveur vanille	Infant cereals. vanilla taste	BPW + α-amylase	st	/	/	-	31.08	34.04	+	19.37	31.81	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a

MICROVAL® NEN

INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																															
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	**Reference method: ISO 21528-1♦**				**Alternative method: iQ-Check *Enterobacteriaceae***																				Category	Type
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																					
									PCR result			PCR result			Confirmation															
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																		
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG											
					Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②		
2024	**1448**	**Poudre de lait infantile 6 mois - 1 an avec probiotiques (*S. thermophilus*: 1.7 x 10^4 UFC/g) (24.3% MG)**	**Infant formula with probiotics (*S. thermophilus*: 1.7 x 10^4 CFU/g) (24.3% fat level)**	BPW 2X + Tween 80	st	/	/	-	28.13	32.03	+	27.39	31.57	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b
2024	**1449**	**Poudre de lait infantile 0-6 mois avec probiotiques (*B. lactis*: 5.3 x 10^3 UFC/g) (28.2% MG)**	**Infant formula with probiotics (*B. lactis*: 5.3 x 10^3 CFU/g) (28.2% fat level)**	BPW 2X + Tween 80	+p	-	+	+	18.79	N/A	+	20.25	31.68	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1450**	**Poudre de lait infantiles dès 6 mois avec probiotiques (L.imosilactobabillus: 1.1 x 10^5 UFC/g) (>20% MG)**	**Infant formula with probiotics (L.imosilactobabillus: 1.1 x 10^5 CFU/g) (>20% fat level)**	BPW 2X + Tween 80	+p	-	+	+	20.01	34.41	+	20.77	31.04	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1451**	**Poudre de lait infantile 6 mois - 1 an avec probiotiques (*L. reuteri*: 3.2 x 10^4 UFC/g) (24.3% MG)**	**Infant formula with probiotics (*L. reuteri*: 3.2 x 10^4 CFU/g) (24.3% fat level)**	BPW 2X + Tween 80	st	/	/	-	N/A	32.66	-	N/A	31.62	-	-C	/	/	-C	/	/	-	-	-	**NA**	**NA**	1	b
2024	**1452**	**Poudre de lait infantile dès 6 mois avec probiotiques (*L. reuteri*: 3.1 x 10^5 UFC/g) (27.6% MG)**	**Infant formula with probiotics (*L. reuteri*: 3.1 x 10^5 CFU/g) (27.6% fat level)**	BPW 2X + Tween 80	+p	-	+	+	25.50	N/A	+	26.57	31.24	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1453**	**Céréales infantiles lentilles, carottes, pois avec probiotiques (*B. lactis*: 2.7 x 10^3 UFC/g)**	**Infant formula with probiotics (*B. lactis*: 2.7 x 10^3 CFU/g)**	BPW 2X + α-amylase	st	/	/	-	31.43	32.65	+	N/A/N/A/N/A	34.59/ 37.82/ 38.78	-/-/-	+p	-	+	+p	-	+	+	+	-	**PD**	**$NA_{FN(alt)}$**	1	b

MICROVAL® NEN

INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																															
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	**Reference method: ISO 21528-1♦**				**Alternative method: iQ-Check *Enterobacteriaceae***																				Category	Type
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																					
									PCR result			PCR result			Confirmation															
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																		
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG											
					Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②		
2024	**1454**	**Céréales infantiles saveur banane avec probiotiques (*B. lactis*: 3.9 x 10^5 UFC/g)**	**Infant formula with probiotics (*B. lactis*: 3.9 x 10^5 CFU/g)**	BPW 2X + α-amylase	st	/	/	-	31.03	32.35	+	N/A/N/ A/N/A	32.04/ 36.44/ 36.13	-/-/-	+p	-	+	+p	-	+	+	+	-	**PD**	**NA$_{FN(alt)}$**	1	b
2024	**1455**	**Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 2.6 10^4 UFC/g)**	**Infant formula with probiotics (Bifidobacteria: 2.6 10^4 CFU/g)**	BPW 2X + α-amylase	st	/	/	-	21.60	37.31	+	22.90	N/A	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b
2024	**1456**	**Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 1.7 10^4 UFC/g)**	**Infant formula with probiotics (Bifidobacteria: 1.7 10^4 CFU/g)**	BPW 2X + α-amylase	+p	-	+	+	N/A	33.05	-	N/A	33.77	-	st	/	/	-C	/	/	-	-	-	**ND**	**ND**	1	b
2024	**1457**	**Céréales infantiles lentilles. pêche. pomme avec probiotiques (*B. lactis*: 1.1 x 10^6 UFC/g)**	**Infant formula with probiotics(*B. lactis*: 1.1 x 10^6 CFU/g)**	BPW 2X + α-amylase	+p	-	+	+	31.06	42.47/36.48/ 36.39/36.14/ 36.01/34.75/ 36.27/35;37	+	25.15	36.11	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1915**	**Poudre de lait infantile 0-6 mois avec probiotiques (*L. reuteri*: 1.1 x 10^7 UFC/g) (27.8% MG)**	**Infant formula with probiotics (*L. reuteri*: 1.1 x 10^7 CFU/g) (27.8% fat level)**	BPW 2X + Tween 80	+p	-	+	+	29.90	31.11	+	28.96	31.36	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1916**	**Poudre de lait infantile bio 10 mois - 3 ans avec probiotiques (*L. reuteri*:1.5 x 10^6 UFC/g) (24% MG)**	**Infant formula with probiotics (*L. reuteri*:1.5 x 10^6 CFU/g) (24% fat level)**	BPW 2X + Tween 80	+p	-	+	+	21.14	31.00	+	22.78	30.95	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b

INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																															
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	**Reference method: ISO 21528-1♦**				**Alternative method: iQ-Check *Enterobacteriaceae***																					Category	Type
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																					
									PCR result			PCR result			Confirmation																
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																			
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG												
					Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②		
2024	**1917**	**Poudre de lait infantile 0-6 mois avec probiotiques (Limocilactobacillus: 6.3 x 10^6 UFC/g) (>20% MG)**	**Infant formula with probiotics (Limocilactobacillus: 6.3 x 10^6 CFU/g) (>20% fat level)**	BPW 2X + Tween 80	+p	-	+	+	N/A	31.58	-	N/A	32.13	-	-C	/	/	st	/	/	-	-	-	**ND**	**ND**	1	b
2024	**1918**	**Céréales infantile pêche/pomme avec probiotiques (*B. lactis*: 1.2 x 10^6 UFC/g)**	**Infant cereals with probiotics (*B. lactis*: 1.2 x 10^6 CFU/g)**	BPW 2X + Tween 80	+p	-	+	+	N/A	35.81	-	N/A	33.49	-	st	/	/	st	/	/	-	-	-	**ND**	**ND**	1	b
2024	**2232**	Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 2.6 x 10^4 UFC/g)	Infant cereals with probiotics (Bifidobacteria: 2.6 x 10^4 CFU/g)	BPW 2X + α-amylase	-A	/	/	-	22.32	34.16	+	28.19	31.61	+	d	-	+	+p	-	+	+ (*Enterobacter cloacae*)	+	+	**PD**	**PD**	1	b
2024	**2233**	Céréales infantiles banane. flocons d'avoine avec probiotiques (*B. lactis* : 3.9 10^5 CFU/g)	Infant cereals with probiotics (*B. lactis* : 3.9 10^5 CFU/g)	BPW 2X + α-amylase	st	/	/	-	N/A	35.94	-	N/A	32.45	-	d	-	-	-	/	/	-	-	-	**NA**	**NA**	1	b
2024	**2234**	Céréales infantiles pomme, pêche, lentilles, flocons d'avoine avec probiotiques (*B. lactis* : 1.0 x 10^8 UFC/g)	Infant cereals with probiotics (*B. lactis* : 1.0 x 10^8 CFU/g)	BPW 2X + α-amylase	d	- x5	- x5	-	34.35	34	+	23.07	31.76	+	+p	-	+	-C	/	/	+ (*Enterobacter cloacae*)	+	+	**PD**	**PD**	1	b
2024	**2235**	Poudre de lait infantile 0 - 6 mois avec probiotiques (*L. reuteri*: 1.1 x 10^7 UFC/g) (27.6% MG)	Infant formula with probiotics (*L. reuteri*: 1.1 x 10^7 CFU/g) (27.6% fat level)	BPW 2X + Tween 80	st	/	/	-	N/A/N/A/N/A	32.55/ 31.71/ 31.66	-/-/-	33.84 /N/A/N/A	32.4/ 32.38/ 32.36	+/-/-	st x5	/	/	st x5	/	/	-	-	-	**NA**	**$PD_{FP(alt)}$**	1	b

INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																														
					Reference method: ISO 21528-1♦				**Alternative method: iQ-Check *Enterobacteriaceae***																					
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																					
									PCR result			PCR result			Confirmation															
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																		
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG											
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type
2024	**2236**	Poudre de lait infantile système immunitaire dès 6 mois 6-12 mois (*L. reuteri*: 7.0 x 10^6 UFC/g) (24% MG)	Infant formula with probiotics (*L. reuteri*: 7.0 x 10^6 CFU/g) (24% fat level)	BPW 2X + Tween 80	st	/	/	-	31.07/ 30.59/ 31.19	32.54/ 32.17/ 32.62	+/+/+	32.25/ 30.19/ 29.73	32.75/ 31.52/ 31.45	+/+/+	st x5	/	/	st x5	/	/	-	-	-	**$PD_{FP(alt)}$**	**$PD_{FP(alt)}$**	1	b
2024	**2237**	Poudre de lait infantile 6-12 mois (*L. infantis*: 5.1 x 10^5 UFC/g) (23% MG)	Infant formula (*L. infantis*: 5.1 x 10^5 CFU/g) (23% fat level)	BPW 2X + Tween 80	st	/	/	-	N/A	32.0	-	N/A	32.34	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	b
2024	**1442**	**Maltodextrine**	**Maltodextrin**	BPW	+p	-	+	+	17.06	33.81	+	20.75	N/A	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1443**	**Lactose**	**Lactose**	BPW	st	/	/	-	20.06	31.35	+	24.47	33.96	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	c
2024	**1444**	**Protéine de lactosérum**	**Whey proteins**	BPW	st	/	/	-	25.39	31.07	+	N/A/ N/A/ N/A/ 27.43*/ 27.33*	31.95/ N/A/ N/A/ N/A/ 31.31/ 31.32	i/i/i/+*/+*	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	c
2024	**1445**	**Caséinate**	**Caseinate**	BPW	st	/	/	-	N/A/N/A/N/A	32.05/ 32.68/ 32;51	-/-/-	N/A	33.26	-/-/-	+p	-	+	+M	-	+	+	-	-	**$NA_{FN(alt)}$**	**$NA_{FN(alt)}$**	1	c
2024	**1446**	**Amidon de blé**	**Wheat starch**	BPW	+p	-	+	+	22.27	32.09	+	26.11	31.43	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1458**	**Lactose**	**Lactose**	BPW	st	/	/	-	36.94	33.10	+	33.88	32.3	+	d	- x5	+ x5	-C	/	/	+	+	+	**PD**	**PD**	1	c
2024	**1459**	**Lactosérum**	**Whey**	BPW	+p	-	+	+	N/A/N/A/N/A	32.57/ 32.72/ 43.18	-/-/-	34.05	32.2	+	+p	-	+	+p	-	+	+	-	+	**$ND_{FN(alt)}$**	**PA**	1	c
2024	**1460**	**Amidon de mais**	**Corn starch**	BPW	st	/	/	-	N/A	33.18	-	N/A	31.92	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	c
2024	**1461**	**Amidon de riz**	**Rice starch**	BPW	+p	-	+	+	18.71	N/A	+	20.85	N/A	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c

MICROVAL® NEN

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Year of analysis	Sample N°	Product (French name)	Product	ISO method Enrichment broth	Reference method: ISO 21528-1♦				Alternative method: iQ-Check *Enterobacteriaceae*																			Category	Type
									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																				
									PCR result			PCR result			Confirmation														
					VRBG	Confirmation			Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																	
									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG										
					Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②		
2024	**1462**	**Amidon de blé**	**Wheat starch**	BPW	+p	-	+	+	18.51	33.38	+	20.10	31.65	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1919**	**Maltodextrine**	**Maltodextrin**	BPW	+p	-	+	+	N/A	32.27	-	N/A	32.79	-	st	/	/	st	/	/	-	-	-	**ND**	**ND**	1	c
2024	**1920**	**Amidon de mais**	**Corn starch**	BPW	st	/	/	-	N/A	32.35	-	N/A	32.74	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	c
2024	**1921**	**Caséinate**	**Caseinate**	BPW	+p	-	+ (E. coli)	+	N/A	31.76	-	N/A	32.48	-	+p	-	- x10	+p	-	- x10	- (16S: *Pseudomonas oryzihabitans*)	-	-	**ND**	**ND**	1	c
2024	**2238**	Amidon de riz	Rice starch	BPW	st	/	/	-	N/A	34.56	-	N/A	32.27	-	-A	/	/	-A	/	/	-	-	-	**NA**	**NA**	1	c
2024	**2240**	Lactosérum	Whey	BPW	st	/	/	-	N/A	35.18	-	N/A	32.25	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	c
2024	**2241**	Amidon de blé	Wheat starch	BPW	-C	/	/	-	15.93	38.66	+	17.16	32.96	+	+p	-	+	-A	/	/	+ (*Enterobacter cloacae*)	+	+	**PD**	**PD**	1	c
2024	**2242**	Lactose	Lactose	BPW	st	/	/	-	N/A	33.0	-	N/A	32.54	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	c
2024	**2243**	Lactosérum	Whey	BPW	st	/	/	-	N/A	32.31	-	N/A	34.19	-	-A	/	/	-A	/	/	-	-	-	**NA**	**NA**	1	c
2024	**2244**	Maltodextrine	Maltodextrin	BPW	st	/	/	-	27.22	31.53	+	36.37	32.98	+	-A	/	/	-A	/	/	- (+ at 72 h : *Serratia plymuthica*)	+	+	**PD**	**PD**	1	c
2024	**2469**	**Poudre de lait écrémé**	**Non-fat level dry milk**	BPW	+p	-	+	+	26.92	32.13	+	30.19	33.42	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**2470**	**Poudre de lait écrémé**	**Non-fat level dry milk**	BPW	+p	-	+	+	21.63	33.61	+	26.27	32.85	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**2471**	**Poudre de lait écrémé**	**Non-fat level dry milk**	BPW	+p	-	+	+	19.85	35.76	+	19.21	N/A	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c

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									1/4 dilution. Pre-warmed BPW + PIF supplement - 18 h at 37 ± 1°C																						
									PCR result			PCR result			Confirmation																
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									CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG												
					Typical colonies	Oxidase test	OF media (glucose fermentation test)	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol ①*	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②		
2024	**2472**	Poudre de lait écrémé	Non-fat level dry milk	BPW	-c	/	/	-	N/A	32.72	-	N/A	32.05	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	c

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						Alternative method: iQ-Check *Enterobacteriaceae*																			
						Pre-warmed BPW + PIF supplement - 18 h at 37 C ± 1°C after storage for 72h at 5 ± 3°C																			
						PCR result			PCR result			Confirmation													
						Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																
						CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG										
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	**Reference method: ISO 21528-1♦** Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol* ①	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type
2024	**1432**	**Poudre de lait infantile dès 6 mois (37% MG)**	**Infant formula (37% fat level)**	BPW + Tween 80	-	N/A	32.48	-	N/A	31.61	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	a
2024	**1433**	**Poudre de lait infantile 6-12 mois bio (23% MG)**	**Infant formula (23% fat level)**	BPW + Tween 80	-	21.13	N/A	+	24.21	31.14	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a
2024	**1434**	**Poudre de lait infantile 6-12 mois (32% MG)**	**Infant formula (32% fat level)**	BPW + Tween 80	+	21.12	33.99	+	25.94	31.21	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1436**	**Poudre de lait infantile dès 6 mois (23.6% MG)**	**Infant formula (23.6% fat level)**	BPW + Tween 80	-	24.85	N/A	+	24.50	31.29	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a
2024	**1437**	**Céréales infantiles saveur biscuit**	**Infant cereals, biscuit taste**	BPW + α-amylase	+	N/A	36.40	-	N/A	32.33	-	st	/	/	st	/	/	-	-	-	**ND**	**ND**	1	a
2024	**1438**	**Céréales infantiles légumes du potager**	**Infant cereals, vegetables**	BPW + α-amylase	-	18.91	N/A	+	22.19	31.03	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a
2024	**1439**	**Céréales infantiles multicéréales/légumes du soleil dès 8 mois**	**Infant cereals, multicereals, vegetables**	BPW + α-amylase	-	24.19	33.07	+	27.27	31.48	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a
2024	**1441**	**Céréales infantiles 3 céréales**	**Infant cereals**	BPW + α-amylase	+	23.06	33.70	+	24.46	31.88	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1910**	**Poudre de lait infantile 0-6 mois (>20% MG)**	**Infant formula**	BPW + Tween 80	+	36.95	N/A	+	19.95	36.46	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1911**	**Poudre de lait infantile 6-125 mois (22% MG)**	**Infant formula**	BPW + Tween 80	+	19.31	40.64	+	19.25	N/A	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1912**	**Poudre de lait infantile 6-12 mois (26% MG)**	**Infant formula**	BPW + Tween 80	+	18.34	35.75	+	18.83	N/A	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1913**	**Céréales infantiles 6-36 mois**	**Infant cereals**	BPW + α-amylase	+	21.11	32.70	+	19.30	33.04	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**1914**	**Céréales infantiles dès 6 mois**	**Infant cereals**	BPW + α-amylase	+	N/A	37.40	-	31.38/N/A/N/A	32.17/ 34.39/ 34.89	+/-/-	st	/	/	-C	/	/	-	-	-	**ND**	**PPND**	1	a
2024	**1922**	**Lactose**	**Lactose**	BPW	+	22.28	31.90	+	24.37	31.18	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	a
2024	**2230**	Céréales infantiles saveur biscuit	Infant cereals. biscuit taste	BPW + α-amylase	+	25.7	32.51	+	26.72	31.09	+	d	- x5	+ x5	d	- x5	+ x5	+(*Pantoea* spp)	+	+	**PA**	**PA**	1	a

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

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Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	**Reference method: ISO 21528-1♦** Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol* ①	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type
2024	**2231**	Céréales infantiles saveur vanille	Infant cereals. vanilla taste	BPW + α-amylase	-	19.44	N/A	+	19.44	N/A	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	a
2024	**1448**	**Poudre de lait infantile 6 mois - 1 an avec probiotiques (*S. thermophilus*: 1.7 x 10^4 UFC/g) (24.3% MG)**	**Infant formula with probiotics (*S. thermophilus*: 1.7 x 10^4 CFU/g) (24.3% fat level)**	BPW 2X + Tween 80	-	22.98	32.16	+	22.60	33.24	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b
2024	**1449**	**Poudre de lait infantile 0-6 mois avec probiotiques (*B. lactis*: 5.3 x 10^3 UFC/g) (28.2% MG)**	**Infant formula with probiotics (*B. lactis*: 5.3 x 10^3 CFU/g) (28.2% fat level)**	BPW 2X + Tween 80	+	18.31	32.47	+	18.65	31.73	+	+M	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1450**	**Poudre de lait infantiles dès 6 mois avec probiotiques (*L.imosilactobabillus*: 1.1 x 10^5 UFC/g) (>20% MG)**	**Infant formula with probiotics (*L.imosilactobabillus*: 1.1 x 10^5 CFU/g) (>20% fat level)**	BPW 2X + Tween 80	+	19.51	31.27	+	20.48	30.93	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1452**	**Poudre de lait infantile dès 6 mois avec probiotiques (*L. reuteri*: 3.1 x 10^5 UFC/g) (27.6% MG)**	**Infant formula with probiotics (*L. reuteri*: 3.1 x 10^5 CFU/g) (27.6% fat level)**	BPW 2X + Tween 80	+	18.81	32.46	+	19.82	32.06	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1453**	**Céréales infantiles lentilles, carottes, pois avec probiotiques (*B. lactis*: 2.7 x 10^3 UFC/g)**	**Infant formula with probiotics (*B. lactis*: 2.7 x 10^3 CFU/g)**	BPW 2X + α-amylase	-	N/A/N/ A/N/A	39.05/ 39.78/ 39.77	-/- /-	35.03	33.40	+	+p	-	+	+p	-	+	+	-	+	$NA_{FN(alt)}$	**PD**	1	b
2024	**1454**	**Céréales infantiles saveur banane avec probiotiques (*B. lactis* : 3.9 x 10^5 UFC/g)**	**Infant formula with probiotics (*B. lactis* : 3.9 x 10^5 CFU/g)**	BPW 2X + α-amylase	-	38.72	33.60	+	34.96	34.29	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b
2024	**1455**	**Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 2.6 x 10^4 UFC/g)**	**Infant formula with probiotics (Bifidobacteria:2.6 x 10^4 CFU/g)**	BPW 2X + α-amylase	-	19.59	32.36	+	21.29	N/A	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b

INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																											
					Reference method: ISO 21528-1♦	**Alternative method: iQ-Check *Enterobacteriaceae***																					
						Pre-warmed BPW + PIF supplement - 18 h at 37 C ± 1°C after storage for 72h at 5 ± 3°C																		
						PCR result			PCR result			Confirmation														
						Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																
						CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG										
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol* ①	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type
2024	**1456**	**Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries: 1.7 x 10^4 UFC/g)**	**Infant formula with probiotics (Bifidobacteria: 1.7 x 10^4 CFU/g)**	BPW 2X + α-amylase	+	33.28	32.74	+	32.99	33.22	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1457**	**Céréales infantiles lentilles, pêche, pomme avec probiotiques (*B. lactis*: 1.1 x 10^6 UFC/g)**	**Infant formula with probiotics(*B. lactis*: 1.1 x 10^6 CFU/g)**	BPW 2X + α-amylase	+	32.66	36.28	+	27.17	31.95	+	+M	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1915**	**Poudre de lait infantile 0-6 mois avec probiotiques (*L. reuteri*:1.1 x 10^7 UFC/g) (27.8% MG)**	**Infant formula with probiotics (*L. reuteri*: 1.1 x 10^7 CFU/g) (27.8% fat level)**	BPW 2X + Tween 80	+	21.56	33.29	+	21.49	32.12	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1916**	**Poudre de lait infantile bio 10 mois - 3 ans avec probiotiques (*L. reuteri*:1.5 x 10^6 UFC/g) (24% MG)**	**Infant formula with probiotics (*L. reuteri*:1.5 x 10^6 CFU/g) (24% fat level)**	BPW 2X + Tween 80	+	19.89	32.52	+	19.52	31.69	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	b
2024	**1917**	**Poudre de lait infantile 0-6 mois avec probiotiques (*Limocilactobacillus*: 6.3 x 10^6 UFC/g) (>20% MG)**	**Infant formula with probiotics (*Limocilactobacillus*: 6.3 x 10^6 CFU/g) (>20% fat level)**	BPW 2X + Tween 80	+	N/A	31.93	-	N/A	32.37	-	-C	/	/	st	/	/	-	-	-	**ND**	**ND**	1	b
2024	**1918**	**Céréales infantile pêche/pomme avec probiotiques (*B. lactis*: 1.2 x 10^6 UFC/g)**	**Infant cereals with probiotics (*B. lactis*: 1.2 x 10^6 CFU/g)**	BPW 2X + Tween 80	+	N/A	43.92	-	N/A	33.68	-	-C	/	/	st	/	/	-	-	-	**ND**	**ND**	1	b
2024	**2232**	Céréales infantiles dès 6 mois avec probiotiques (Bifidobactéries:2.6 x 10^4 UFC/g)	Infant cereals with probiotics (Bifidobactéria:2.6 x 10^4 CFU/g)	BPW 2X + α-amylase	-	24.9	34.4	+	18.24	31.42	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b
2024	**2234**	Céréales infantiles pomme, pêche, lentilles, flocons d'avoine avec probiotiques (*B. lactis* :1.0 x 10^8 UFC/g)	Infant cereals with probiotics (*B. lactis* :1.0 x 10^8 CFU/g)	BPW 2X + α-amylase	-	21.96	36.46	+	18.18	31.48	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	b

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INFANT FORMULA, INFANT CEREALS WITH OR WITHOUT PROBIOTICS AND RELATED INGREDIENTS																											
					Reference method: ISO 21528-1♦	**Alternative method: iQ-Check *Enterobacteriaceae***																					
						Pre-warmed BPW + PIF supplement - 18 h at 37 C ± 1°C after storage for 72h at 5 ± 3°C																		
						PCR result			PCR result			Confirmation														
						Easy I lysis with FDRS (protocol ①)			1/10 sample Easy I lysis with FDRS (protocol ②)																
						CFX OPUS			CFX OPUS			RAPID'*Enterobacteriaceae*			VRBG										
Year of analysis	**Sample N°**	**Product (French name)**	**Product**	ISO method Enrichment broth	Final result	Cq	IC Cq	Result *Enterobacteriaceae*	Cq	IC Cq	Result *Enterobacteriaceae*	Typical colonies	Oxidase test	OF media (glucose fermentation test)	Typical colonies	Oxidase test	OF media (glucose fermentation test)	All confirmation tests	Final result *protocol* ①	Final result protocol ②	Agreement Ref/Alt ①	Agreement Ref/Alt ②	Category	Type
2024	**2235**	Poudre de lait infantile 0 - 6 mois avec probiotiques (*L. reuteri*: 1.1 x 10^7 UFC/g) (27.6% MG)	Infant formula with probiotics (*L. reuteri*: 1.1 x 10^7 CFU/g) (27.6% fat level)	BPW 2X + Tween 80	-	N/A	32.5	-	36.1/ 33.07/ 33.76	31.7/ 31.62/ 31.97	+/+/+	st x5	/	/	st x5	/	/	-	-	-	**NA**	**$PD_{FP(alt)}$**	1	b
2024	**2236**	Poudre de lait infantile système immunitaire dès 6 mois 6-12 mois (*L. reuteri*:7.0 x 10^6 UFC/g) (24% MG)	Infant formula with probiotics (*L. reuteri*:7.0 x 10^6 CFU/g) (24% fat level)	BPW 2X + Tween 80	-	N/A	32.54	-	32.61/ 29.9/ 30.26	31.47/ 31.43/ 31.55	+/+/+	st x5	/	/	st x5	/	/	-	-	-	**NA**	**$PD_{FP(alt)}$**	1	b
2024	**1442**	**Maltodextrine**	**Maltodextrin**	BPW	+	18.23	N/A	+	21.62	31.07	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1443**	**Lactose**	**Lactose**	BPW	-	19.60	31.27	+	24.07	31.14	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	c
2024	**1444**	**Protéine de lactoserum**	**Whey proteins**	BPW	-	25.88	30.82	+	N/A/N/A/ N/A/27.43*/ 27.33*	31.95/N/A/ N/A/31.31/ 31.32	- /i/i/+*/+*	+p	-	+	+p	-	+	+	+	-	**PD**	**NA**	1	c
2024	**1445**	**Caséinate**	**Caseinate**	BPW	-	N/A	32.09	-	N/A	31.80	-	+M	-	+	+1/2	-	+	+	-	-	**$NA_{FN(alt)}$**	**$NA_{FN(alt)}$**	1	c
2024	**1446**	**Amidon de blé**	**Wheat starch**	BPW	+	23.76	31.32	+	24.73	31.16	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1458**	**Lactose**	**Lactose**	BPW	-	29.15	31.74	+	38.25	31.93	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	c
2024	**1459**	**Lactoserum**	**Whey**	BPW	+	N/A/N/ A/N/A	31.93/ 32.58/ 32.58	-/- /-	37.65	32.22	+	+p	-	+	+p	-	+	+	-	+	**$ND_{FN(alt)}$**	**PA**	1	c
2024	**1461**	**Amidon de riz**	**Rice starch**	BPW	+	18.69	33.50	+	20.62	32.55	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1462**	**Amidon de blé**	**Wheat starch**	BPW	+	17.32	N/A	+	18.73	N/A	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**1919**	**Maltodextrine**	**Maltodextrin**	BPW	+	N/A	33.44	-	N/A	32.32	-	st	/	/	st	/	/	-	-	-	**ND**	**ND**	1	c
2024	**1920**	**Amidon de mais**	**Corn starch**	BPW	-	N/A	33.20	-	N/A	32.50	-	st	/	/	st	/	/	-	-	-	**NA**	**NA**	1	c
2024	**1921**	**Caséinate**	**Caseinate**	BPW	+	N/A	32.22	-	N/A	32.45	-	+p	-	-	+p	-	-	- x5	-	-	**ND**	**ND**	1	c
2024	**2241**	Amidon de blé	Wheat starch	BPW	-	16.2	N/A	+	18.55	31.4	+	+p	-	+	+p	-	+	+	+	+	**PD**	**PD**	1	c
2024	**2244**	Maltodextrine	Maltodextrin	BPW	-	16.81	21.47	+	31.83	31.6	+	d	- x5	+ x5	d	- x5	+ x5	+ (*Serratia plymuthica*)	+	+	**PD**	**PD**	1	c
2024	**2469**	**Poudre de lait écrémé**	**Non fat level dry milk**	BPW	+	25.98	31.56	+	25.71	31.21	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**2470**	**Poudre de lait écrémé**	**Non fat level dry milk**	BPW	+	21.08	34.40	+	22.45	31.21	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c
2024	**2471**	**Poudre de lait écrémé**	**Non fat level dry milk**	BPW	+	21.53	34.20	+	21.61	31.15	+	+p	-	+	+p	-	+	+	+	+	**PA**	**PA**	1	c

Appendix 6 - RLOD: raw data

Matrix : Infant formula with probiotics
Strain : *Enterobacter Kobei* Ad706
Lactic flora :4,09.10^5 CFU/g

N° sample	Level	Contamination level (CFU/test portion)	Reference method: ISO 21528-1♦			Number positive samples/Total	Alternative method: iQ-Check *Enterobacteriaceae* 1:4 dilution, Pre-warmed BPW + PIF supplement - 18 h-at 37°C ± 1°C										Number positive samples/Total	
							PCR result - CFX OPUS						Confirmation			Final result *Enterobacteriaceae*	Final result *Enterobacteriaceae* 1/10	
							Easy I lysis with FDRS			1/10 sample - Easy I lysis with FDRS								
			VRBG Typical colonies	Confirmation	Result *Enterobacteriaceae*		Cq	IC Cq	Result	Cq	IC Cq	Result	RAPID' *Enterobacteriaceae* Typical colonies	VRBG Typical colonies	All confirmation tests			Protocol ①
346	/	0	st	-	-	0/5	N/A	32.68	-	N/A	32.07	-	st	st	-	-	-	0/5
347			st	-	-		N/A	31.50	-	N/A	31.95	-	st	st	-	-	-	
348			st	-	-		N/A	32.10	-	37.07/i/ 33.30	31.94/i/ 31.72	+/-/+	st	st	-	-	-	
349			st	-	-		N/A	32.51	-	N/A	32.51	-	st	st	-	-	-	
350			st	-	-		N/A	32.71	-	38.27/ 34,01/ 34.11	31.88/ 31.32/ 31.58	+/+/+	st	st	-	-	-	
351	Low	1,6	+p	+	+	13/20	N/A	32.29	-	N/A	33.03	-	st	st	-	-	-	12/20
352			+p	+	+		23.39	34.37	+	22.43	31.22	+	+p	+p	+	+	+	
353			+p	+	+		29.13	31.32	+	23.00	31.32	+	+p	+p	+	+	+	
354			+p	+	+		24.62	31.80	+	23.89	30.84	+	+p	+p	+	+	+	
355			st	-	-		N/A	33.28	-	N/A	32.10	-	st	st	-	-	-	
356			+p	+	+		N/A	32.00	-	N/A	32.14	-	st	st	-	-	-	
357			+p	+	+		23.78	36.86	+	25.88	31.27	+	+p	+p	+	+	+	
358			+p	+	+		30.51	31.02	+	23.50	31.14	+	+p	+p	+	+	+	
359			+p	+	+		N/A/ 23.44/ 24.27	32.65/ 30.91/ 32.41	-/+/+	23.42	31.73	+	+p	+p	+	-	+	
360			st	-	-		29.55	34.11	+	23.16	31.54	+	+p	+p	+	+	+	
361			+p	+	+		N/A	32.86	-	N/A	32.18	-	st	st	-	-	-	
362			st	-	-		N/A	32.51	-	37.36/ N/A/ 34.41	32.17/3 3.05/ 31.61	+/-/+	st	st	-	-	-	
363			+p	+	+		24.32	32.46	+	24.83	31.34	+	+p	+p	+	+	+	
364			+p	+	+		28.76	31.93	+	25.03	31.70	+	+p	+p	+	+	+	
365			st	-	-		N/A	31.87	-	N/A	32.92	-	st	st	-	-	-	
366			st	-	-		24.93	32.46	+	23.50	31.51	+	+p	+p	+	+	+	
367			+p	+	+		23.93	32.07	+	22.90	31.85	+	+p	+p	+	+	+	
368			st	-	-		22.95	31.15	+	23.78	31.50	+	+p	+p	+	+	+	
369			st	-	-		23.11	33.94	+	24.38	31.43	+	+p	+p	+	+	+	
370			+p	+	+		N/A	32.15	-	N/A	31.91	-	st	st	-	-	-	
371	High	4,8	+p	+	+	3/5	24.40	32.02	+	24.07	30.92	+	+p	+p	+	+	+	5/5
372			st	-	-		25.23	31.85	+	23.74	31.07	+	+p	+p	+	+	+	
373			st	-	-		22.43	31.92	+	23.98	30.97	+	+p	+p	+	+	+	
374			+p	+	+		24.29	30.62	+	25.39	31.41	+	+p	+p	+	+	+	
375			+p	+	+		25.84	31.84	+	23.87	31.05	+	+p	+p	+	+	+	

N° sample	Number positive samples/Total Protocol ②
346	0/5
347	
348	
349	
350	
351	13/20
352	
353	
354	
355	
356	
357	
358	
359	
360	
361	
362	
363	
364	
365	
366	
367	
368	
369	
370	
371	5/5
372	
373	
374	
375	

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

Appendix 7 - Inclusivity and exclusivity: raw data

INCLUSIVITY													
N°	Family	Genus	Species	Reference	Origin	Inoculation level (CFU/225ml)	iQ-Check EB			Confirmation			
							CFX OPUS (with FDRS)			RAPID' *Enterobacteriaceae*	VRBG	OF glucose	Oxidase
							Cq	IC Cq	Results				
1	*Enterobacteriaceae*	*Buttiauxella*	*agrestis*	Ad2850	Dairy industry environment	23	16.46	35.57	+	+	+	+	-
2	*Enterobacteriaceae*	*Citrobacter*	*werkmanii*	Ad3004	Raw milk	19	17.67	31.18	+	+	+	+	-
3	*Enterobacteriaceae*	*Citrobacter*	*farmeri*	Ad1116	Environmental sample	14	16.67	35.35	+	+	+	+	-
4	*Enterobacteriaceae*	*Citrobacter*	*youngae*	Ad1372	Water	41	17.51	31.71	+	+	+	+	-
5	*Enterobacteriaceae*	*Cronobacter*	*muytjensii*	CIP103581	/	31	16.83	33.66	+	+ (green)	+ (yellow)	+	-
6	*Enterobacteriaceae*	*Cronobacter*	*malonaticus*	DSM18702	Milk powder	23	16.94	N/A	+	+ (green in the mass, red when isolated)	+ (yellow in the mass, red when isolated)	+	-
7	*Enterobacteriaceae*	*Cronobacter*	*turicensis*	Ad1445	Infant formula	28	17.49	32.44	+	+ (green)	+ (yellow)	+	-
8	*Enterobacteriaceae*	*Cronobacter*	*sakazakii*	Ad1446	Infant formula	80	15.73	N/A	+	+ (green)	+ (yellow)	+	-
9	*Enterobacteriaceae*	*Cronobacter*	*condimenti*	LMG26250T	Spicy meat	23	17.35	32.48	+	+ (green)	+ (yellow)	+	-
10	*Enterobacteriaceae*	*Cronobacter*	*dublinensis*	DSM18705	Milk powder	19	19.62	31.32	+	+ (green)	+ (yellow)	+	-
11	*Enterobacteriaceae*	*Cronobacter*	*universalis*	NCTC9529T	Water	39	17.78	32.30	+	+ (green)	+ (yellow)	+	-
12	*Enterobacteriaceae*	*Enterobacter*	*cloacae*	Ad230	Tuna	25	17.36	31.40	+	+ (green/orange)	+ (yellow/red)	+	-
13	*Enterobacteriaceae*	*Enterobacter*	*kobei*	Ad342	Ham	16	19.52	30.60	+	+ (green/orange)	+ (yellow)	+	-
14	*Enterobacteriaceae*	*Enterobacter*	*aerogenes*	Ad889	Meat flour	6	17.46	31.21	+	+ (green/orange)	+ (yellow)	+	-
15	*Enterobacteriaceae*	*Enterobacter*	*hormaechei*	Ad990	Butter	8	17.19	31.40	+	+ (green/orange)	+ (yellow in the mass, red when isolated)	+	-
16	*Enterobacteriaceae*	*Enterobacter*	*helveticus*	DSM18396	/	9	16.16	33.11	+	+	+	+	-
17	*Enterobacteriaceae*	*Escherichia*	*vulneris*	Ad2853	Dairy industry environment	21	17.07	33.55	+	+	+	+	-
18	*Enterobacteriaceae*	*Escherichia*	*hermanii*	Ad457	Spinach	11	18.99	31.20	+	+	+	+	-
19	*Enterobacteriaceae*	*Escherichia*	*fergusonii*	Ad1381	Process water	37	19.20	31.11	+	+	+	+	-
20	*Enterobacteriaceae*	*Escherichia*	*coli*	Ad1422	Infant formula	22	18.70	31.08	+	+	+	+	-
21	*Enterobacteriaceae*	*Klebsiella*	*oxytoca*	Ad1509	Milk powder	25	17.68	30.90	+	+ (green)	+ (yellow)	+	-
22	*Enterobacteriaceae*	*Klebsiella*	*pneumoniae*	Ad1369	Water	16	17.04	33.66	+	+ (green/orange)	+ (yellow)	+	-
23	*Enterobacteriaceae*	*Kluyvera*	*ascorbata*	CIP82.95T	/	9	17.63	32.06	+	+	+	+	-
24	*Enterobacteriaceae*	*Kluyvera*	*intermedia*	Ad3003	Raw milk	34	19.03	30.61	+	+	+	+	-
25	*Enterobacteriaceae*	*Leclercia*	*adecarboxylata*	Ad707	Milk powder	34	16.65	31.65	+	+	+	+	-
26	*Enterobacteriaceae*	*Lelliotia*	*ammigena*	A00C068	Cockerel	16	18.13	31.38	+	+ (green)	+ (brown in the mass)	+	-
27	*Enterobacteriaceae*	*Raoultella*	*terrigena*	Ad1370	Water process	9	17.83	31.96	+	+ (green in the mass)	+	+	-
28	*Enterobacteriaceae*	*Salmonella*	*Diarizonae* 38: lv: z53	Ad451	Ewe milk cheese	34	19.88	31.81	+	+	+	+	-
29	*Enterobacteriaceae*	*Salmonella*	*Typhimurium*	Ad1070	Pork meat	25	22.08	31.02	+	+	+	+	-
30	*Enterobacteriaceae*	*Salmonella*	*Salamae* 42:b:enxz15	Ad593	Seeds	18	20.97	32.71	+	+	+	+	-
31	*Enterobacteriaceae*	*Salmonella*	*houtenae* 6,14:24,z23:-	Ad1834	Raw ewe milk	23	19.54	30.86	+	+	+	+	-
32	*Enterobacteriaceae*	*Salmonella*	*Indica* 11:b:e,n,x	Ad2337	Environmental chicken farm	30	17.89	31.79	+	+	+	+	-

INCLUSIVITY													
N°	Family	Genus	Species	Reference	Origin	Inoculation level (CFU/225ml)	iQ-Check EB			Confirmation			
							CFX OPUS (with FDRS)			RAPID' *Enterobacteriaceae*	VRBG	OF glucose	Oxidase
							Cq	IC Cq	Results				
33	*Enterobacteriaceae*	*Salmonella*	*arizonae* 50:z4, z23:-	CIP 5526	Egg product	13	20.18	31.00	+	+	+	+	-
34	*Enterobacteriaceae*	*Shigella*	*sonnei*	CIP82.49T	/	14	17.44	N/A	+	+	+	+	-
35	*Enterobacteriaceae*	*Shigella*	*flexneri*	Ad2002	Meat product	47	18.90	32.03	+	+	++	+	-
36	*Enterobacteriaceae*	*Shimwellia*	*Blattae*	ATCC29907		17	17.50	N/A	+	+	+	+	-
37	*Budviciaceae*	*Leminorella*	*richardii*	DSM14849	Clinical (Human faeces)	25	28.03/ 21.30[1] 21.76[2]	31.26	+	+ (translucid)	+	+	-
38	*Morganellaceae*	*Morganella*	*morganii*	CIPA236	/	11	18.07	37.33	+	+	+	+	-
39	*Morganellaceae*	*Proteus*	*mirabilis*	Ad639	Mayonnaise	42	16.95	N/A	+	+	+	+	-
40	*Morganellaceae*	*Proteus*	*vulgaris*	Ad984	Meat product	34	19.40	31.54	+	+	+	+	-
41	*Morganellaceae*	*Providencia*	*stuartii*	Ad1575	River water	16	19.50	32.29	+	+ (green in the mass)	+	+	-
42	*Hafniaceae*	*Edwardsiella*	*tarda*	DSM30052	Clinical (Human faeces)	33	18.78	31.54	+	+	+	+	-
43	*Hafniaceae*	*Hafnia*	*alvei*	Ad1751	Cheese	28	18.45	32.00	+	+	+	+	-
44	*Yersiniaceae*	*Serratia*	*marcescens*	Ad447	Raw milk	30	18.28	32.42	+	+ (green)	+ (brown with pink center)	+	-
45	*Yersiniaceae*	*Serratia*	*fonticola*	Ad1696	Salmon	0	33.64 16.41[1] 20.41[2]	31.63	+	+	+	+	-
46	*Yersiniaceae*	*Yersinia*	*enterocolitica*	Ad1028	Meat product	12	18.53	32.29	+	+	+	+	-
47	*Yersiniaceae*	*Yersinia*	*intermedia*	Adria 33	Milk	23	17.40	33.95	+	+ (green in the mass)	+	+	-
48	*Pectobacteriaceae*	*Pectobacterium*	*carotovorum*	CIP82.83T	/	0	27.28 21.25[1] 22.09[2]	31.08	+	+	no growth	+	-
49	*Erwiniaceae*	*Pantoea*	*agglomerans*	Ad3070	Carrot	8	22.66	31.02	+	+	+ (brown with pink center)	+	-
50	*Erwiniaceae*	*Mixta*	*gaviniae*	DSM22758	Powdered infant formula	28	19.25	33.08	+	+ (green)	+ (brown with pink center)	+	-

[1] Second testing protocol ①
[2] Second testing protocol ②

EXCLUSIVITY								
N°	Genus	species	Reference	Origin	Inoculation level (CFU/225ml)	iQ-Check EB		
						CFX OPUS (with FDRS)		
						Cq	IC Cq	Results
1	*Acinetobacter*	*Baumanii*	Ad2855	Infant formula	1.2×10^5	N/A	30.80	-
2	*Acinetobacter*	*calcoaceticus*	Ad1092	poultry	6.0×10^4	N/A	31.28	-
3	*Acinetobacter*	*johnsonii*	Ad1317	eggs	1.12×10^5	N/A	31.31	-
4	*Acinetobacter*	*spp*	Ad435	Unknown (milk industry)	$4.0;10^3$	N/A	31.65	-
5	*Aeromonas*	*salmonicida*	Ad1716	Egg product	2.4×10^5	N/A	30.83	-
6	*Aeromonas*	*punctata*	Ad1589	Water	1.8×10^5	N/A	31.26	-
7	*Agrobacterium*	*rhizogenes*	CFB1935	Environment	8.0×10^4	N/A	30.98	-
8	*Agrobacterium*	*tumefaciens*	A136	Environment	8.0×10^3	N/A	31.09	-
9	*Arcobacter*	*cryaerophilus*	Ad1124	Chicken	5.8×10^4	N/A	31.85	-
10	*Arcobacter*	*butzleri*	Ad1126	Environment	6.0×10^4	N/A	31.52	-
11	*Burkholderia*	*cepacia*	Ad1541	Environment	4.4×10^4	36.75/N/A/N/A	31.28	+/-/-
12	*Burkholderia*	*vietnamiensis*	Ad1538	Environment	1.2×10^4	N/A	31.03	-
13	*Comamonas*	*aquatica*	Ad1543	Water	2.0×10^3	N/A	31.03	-
14	*Flavobacterium*	*hydratis*	Ad1323	Egg product	2.7×10^5	N/A	31.28	-
15	*Gluconobacter*	*cerinus*	Ad374	Drink	3×10^4	N/A	32.01	-
16	*Gluconobacter*	*oxydans*	Ad997	Drink	1.5×10^3	N/A	31.69	-
17	*Myroides*	*odoratiminus*	Ad1341	Egg product	1.7×10^3	N/A	31.25	-
18	*Ochrobactrum*	*pseudintermedius*	Ad1057	Turkey	4.0×10^3	N/A	31.46	-
19	*Pandoraea*	*sp*	Ad1882	Process water	3.2×10^4	N/A	31.27	-
20	*Paracoccus*	*Yeei*	Ad3061	Vegetable	2.0×10^3	N/A	31.24	-
21	*Pseudomonas*	*otitidis*	Ad1880	Turkey skin	3.4×10^4	N/A	32.35	-
22	*Pseudomonas*	*fluorescens*	Ad1691	Salmon	2.4×10^4	N/A	31.34	-
23	*Pseudomonas*	*aeruginosa*	Ad2307	Egg product	1.4×10^5	N/A	31.56	-
24	*Pseudomonas*	*koreensis*	Ad3067	Broccolis	1.8×10^4	N/A	31.07	-
25	*Psychrobacter*	*psychrophilus*	Ad1343	Eggs	2.1×10^6	N/A	31.34	-
26	*Psychrobacter*	*alimentarius*	Ad2570	Cheese	3.2 x 103	N/A	31.59	-
27	*Ralstonia*	*mannitolilytica*	Ad1059	Turkey skin	1.2×10^4	N/A	31.17	-
28	*Shewanella*	*baltica*	Ad1694	Shrimp	1.3×10^5	N/A	31.20	-
29	*Shingobacterium*	*sp*	Ad1324	Egg product	8.2×10^4	N/A	31.24	-
30	*Pseudochrobactrum*	*sp*	Ad2923	Spiruline	3.7×10^3	N/A	31.77	-

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Appendix 8 - Inter-laboratory study n°2: raw data

Laboratory **A1**
Lactic acid bacteria: 0 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
		Confirmation			PCR result			Confirmation						
	VRBG				DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG					
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)	Reference final result	*Enterobacteriaceae* Cq	IC Cq	PCR final result	Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)	Confirmation final result	Final result	
2	-	/	/	-	N/A	31.49	-	-*	-*	/	/	-	-	**NA**
5	-	/	/	-	N/A	31.89	-	-*	-*	/	/	-	-	**NA**
6	+	-	+	+	N/A	31.63	-	-*	-*	/	/	-	-	**ND**
9	-	/	/	-	N/A	31.6	-	-*	-*	/	/	-	-	**NA**
14	-	/	/	-	N/A	31.67	-	-*	-*	/	/	-	-	**NA**
19	-	/	/	-	N/A	31.51	-	-*	-*	/	/	-	-	**NA**
21	-	/	/	-	N/A	31.02	-	-*	-*	/	/	-	-	**NA**
24	-	/	/	-	N/A	31.73	-	-*	-*	/	/	-	-	**NA**
3	+	-	+	+	18.31	38.56	+	+	+	-	+	+	+	**PA**
8	+	-	+	+	N/A	31.54	-	-*	-*	/	/	-	-	**ND**
11	+	-	+	+	34.38	31.46	+	+	+	-	+	+	+	**PA**
12	+	-	+	+	N/A	31.56	-	-*	-*	/	/	-	-	**ND**
15	+	-	+	+	19.06	36.33	+	+	+	-	+	+	+	**PA**
17	+	-	+	+	N/A	31.57	-	-*	-*	/	/	-	-	**ND**
20	-	/	/	-	17.89	N/A	+	+	+	-	+	+	+	**PD**
22	+	-	+	+	N/A	31.26	-	-*	-*	/	/	-	-	**ND**
1	+	-	+	+	19.2	32	+	+	+	-	+	+	+	**PA**
4	-	/	/	-	17.83	33.33	+	+	+	-	+	+	+	**PD**
7	+	-	+	+	17.45	N/A	+	+	+	-	+	+	+	**PA**
10	+	-	+	+	18.33	N/A	+	+	+	-	+	+	+	**PA**
13	+	-	+	+	N/A	31.55	-	-*	-*	/	/	-	-	**ND**
16	+	-	+	+	17.2	N/A	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	19.75	37.09	+	+	+	-	+	+	+	**PA**
23	+	-	+	+	17.86	35.85	+	+	+	-	+	+	+	**PA**

* Analysis performed 5 days after enrichment

Laboratory **A2**
Lactic acid bacteria: 0 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
25	-	/	/	-	N/A	32.16	-	-*	-*	/	/	-	-	**NA**
28	-	/	/	-	N/A	31.75	-	-*	-*	/	/	-	-	**NA**
30	-	/	/	-	N/A	30.9	-	-*	-*	/	/	-	-	**NA**
35	-	/	/	-	N/A	30.99	-	-*	-*	/	/	-	-	**NA**
38	-	/	/	-	N/A	30.89	-	-*	-*	/	/	-	-	**NA**
41	-	/	/	-	33.05/N/A/N/A	31.07/30.35/30.50	+/-/-	-	-	/	/	-	-	**$PD_{FP(alt)}$**
44	-	/	/	-	N/A	31.29	-	-*	-*	/	/	-	-	**NA**
47	-	/	/	-	N/A	31.96	-	-*	-*	/	/	-	-	**NA**
27	+	-	+	+	N/A	31.05	-	-*	-*	/	/	-	-	**ND**
32	-	/	/	-	18.05	33.89	+	+	+	-	+	+	+	**PD**
33	+	-	+	+	18.55	31.47	+	+	+	-	+	+	+	**PA**
37	-	/	/	-	N/A	31	-	-*	-*	/	/	-	-	**NA**
39	+	-	+	+	18.76	34.27	+	+	+	-	+	+	+	**PA**
42	+	-	+	+	17.54	31.98	+	+	+	-	+	+	+	**PA**
46	+	-	+	+	N/A	31.67	-	-*	-*	/	/	-	-	**ND**
48	-	/	/	-	19.05	N/A	+	+	+	-	+	+	+	**PD**
26	+	-	+	+	19.59	32.99	+	+	+	-	+	+	+	**PA**
29	-	/	/	-	18.27	37.29	+	+	+	-	+	+	+	**PD**
31	+	-	+	+	18.6	28.84	+	+	+	-	+	+	+	**PA**
34	-	/	/	-	17.95	N/A	+	+	+	-	+	+	+	**PD**
36	+	-	+	+	N/A	31.05	-	-*	-*	/	/	-	-	**ND**
40	+	-	+	+	17.62	N/A	+	+	+	-	+	+	+	**PA**
43	-	/	/	-	18.27		+	+	+	-	+	+	+	**PD**
45	+	-	+	+	17.52	N/A	+	+	+	-	+	+	+	**PA**

* Analysis performed 5 days after enrichment

Laboratory **B1** Ref 18h
Lactic acid bacteria: 0 CFU/g Alt 24h CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check Enterobacteriaceae									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG					
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)			
2	-	/	/	-	N/A	30.78	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	22.07	33.27	+	+	+	-	+	+	+	**PD**
6	-	/	/	-	N/A	31.15	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	30.42	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	31.05	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	30.34	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.44	-	-	-	/	/	-	-	**NA**
24	+	-	+	+	N/A	30.92	-	-	-	/	/	-	-	**ND**
3	+	-	+	+	22.96	31.39	+	+	+	-	+	+	+	**PA**
8	+	-	+	+	17.06	31.99	+	+	+	-	+	+	+	**PA**
11	+	-	+	+	18.25	30.80	+	+	+	-	+	+	+	**PA**
12	+	-	+	+	N/A	30.44	-	-	-	/	/	-	-	**ND**
15	+	-	+	+	18.28	35.90	+	+	+	-	+	+	+	**PA**
17	-	/	/	-	18.04	30.72	+	+	+	-	+	+	+	**PD**
20	+	-	+	+	20.65	29.61	+	+	+	-	+	+	+	**PA**
22	+	-	+	+	N/A	30.59	-	-	-	/	/	-	-	**ND**
1	+	-	+	+	22.65	30.31	+	+	+	-	+	+	+	**PA**
4	+	-	+	+	18.09	N/A	+	+	+	-	+	+	+	**PA**
7	+	-	+	+	17.70	N/A	+	+	+	-	+	+	+	**PA**
10	+	-	+	+	18.06	31.04	+	+	+	-	+	+	+	**PA**
13	-	/	/	-	N/A	30.38	-	-	-	/	/	-	-	**NA**
16	+	-	+	+	25.65	32.12	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	18.72	30.62	+	+	+	-	+	+	+	**PA**
23	-	/	/	-	21.09	31.33	+	+	+	-	+	+	+	**PD**

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Laboratory **B2** Ref 18h
Lactic acid bacteria: 0 CFU/g Alt 24h CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG					
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)			
25	+	-	+	+	N/A	30.43	-	+	-	-	+	+	-	$ND_{FN(alt)}$
28	-	/	/	-	N/A	30.69	-	-	-	/	/	-	-	**NA**
30	-	/	/	-	N/A	30.51	-	-	-	/	/	-	-	**NA**
35	-	/	/	-	N/A	30.28	-	-	-	/	/	-	-	**NA**
38	-	/	/	-	N/A	30.28	-	-	-	/	/	-	-	**NA**
41	-	/	/	-	N/A	30.43	-	-	-	/	/	-	-	**NA**
44	-	/	/	-	N/A	30.57	-	-	-	/	/	-	-	**NA**
47	-	/	/	-	30.82	32.05	+	+	+	-	+	+	+	**PD**
27	+	-	+	+	N/A/N/A*/N/A*	30.61	-	+/+**	+/+*	-	+	+	-	$ND_{FN(alt)}$
32	-	/	/	-	N/A	30.35	-	-	-	/	/	-	-	**NA**
33	+	-	+	+	26.72	30.11	+	+	+	-	+	+	+	**PA**
37	-	/	/	-	N/A	30.34	-	-	-	/	/	-	-	**NA**
39	-	/	/	-	N/A	30.29	-	-	-	/	/	-	-	**NA**
42	+	-	+	+	35.89	30.38	+	-	+	-	+	+	+	**PA**
46	-	/	/	-	21.52	N/A	+	+	+	-	+	+	+	**PD**
48	+	-	+	+	17.56	31.66	+	+	+	-	+	+	+	**PA**
26	+	-	+	+	20.09	30.64	+	+	+	-	+	+	+	**PA**
29	+	-	+	+	19.62	30.44	+	+	+	-	+	+	+	**PA**
31	+	-	+	+	19.63	31.32	+	+	+	-	+	+	+	**PA**
34	+	-	+	+	26.09	30.02	+	+	+	-	+	+	+	**PA**
36	-	/	/	-	20.22	30.16	+	+	+	-	+	+	+	**PD**
40	-	/	/	-	19.56	31.63	+	+	+	-	+	+	+	**PD**
43	+	-	+	+	N/A	30.48	-	-	-	/	/	-	-	**ND**
45	+	-	+	+	18.77	30.05	+	+	+	-	+	+	+	**PA**

Laboratory **C1**
Lactic acid bacteria: 0 CFU/g CFX Opus DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check Enterobacteriaceae								Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG				
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)	Confirmation final result	
2	-	/	/	-	N/A	30.61	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	30.53	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	30.71	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	30.79	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	30.53	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	30.84	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.62	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	30.57	-	-	-	/	/	-	-	**NA**
3	+	-	+	+	26.63	30.14	+	+	+	-	+	+	+	**PA**
8	-	/	/	-	N/A	30.65	-	-	-	/	/	-	-	**NA**
11	-	/	/	-	31.28	30.37	+	+	+	-	+	+	+	**PD**
12	+	-	+	+	N/A	30.62	-	-	-	/	/	-	-	**ND**
15	-	/	/	-	N/A/N/A*/N/A*	30.41/30.83*/30.52*	-/-/-	+	+	-	+	+	-	**$NA_{FN(alt)}$**
17	+	-	+	+	24.87	30.12	+	+	+	-	+	+	+	**PA**
20	-	/	/	-	27.51	30.14	+	+	+	-	+	+	+	**PD**
22	+	-	+	+	32.34	30.30	+	+	+	-	+	+	+	**PA**
1	+	-	+	+	33.71	30.68	+	+	+	-	+	+	+	**PA**
4	-	/	/	-	30.61	30.34	+	+	+	-	+	+	+	**PD**
7	+	-	+	+	23.02	30.01	+	+	+	-	+	+	+	**PA**
10	+	-	+	+	22.36	30.19	+	+	+	-	+	+	+	**PA**
13	-	/	/	-	18.34	30.66	+	+	+	-	+	+	+	**PD**
16	+	-	+	+	29.48	30.56	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	21.25	30.10	+	+	+	-	+	+	+	**PA**
23	+	-	+	+	20.44	29.92	+	+	+	-	+	+	+	**PA**

Laboratory **C2**
Lactic acid bacteria: 0 0 CFU/g CFX Opus DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*								Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG				
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)	Confirmation final result	
25	-	/	/	-	N/A	30.45	-	-	-	/	/	-	-	**NA**
28	-	/	/	-	N/A	30.70	-	-	-	/	/	-	-	**NA**
30	-	/	/	-	N/A	30.63	-	-	-	/	/	-	-	**NA**
35	-	/	/	-	N/A	30.54	-	-	-	/	/	-	-	**NA**
38	-	/	/	-	N/A	30.69	-	-	-	/	/	-	-	**NA**
41	-	/	/	-	N/A	30.60	-	-	-	/	/	-	-	**NA**
44	-	/	/	-	N/A	30.49	-	-	-	/	/	-	-	**NA**
47	-	/	/	-	N/A	30.80	-	-	-	/	/	-	-	**NA**
27	+	-	+	+	18.32	30.52	+	+	+	-	+	+	+	**PA**
32	+	-	+	+	N/A	30.62	-	-	-	/	/	-	-	**ND**
33	-	/	/	-	25.98	30.17	+	+	+	-	+	+	+	**PD**
37	-	/	/	-	18.63	30.64	+	+	+	-	+	+	+	**PD**
39	-	/	/	-	18.67	31.08	+	+	+	-	+	+	+	**PD**
42	+	-	+	+	32.71	30.60	+	+	+	-	+	+	+	**PA**
46	+	-	+	+	N/A	30.75	-	-	-	/	/	-	-	**ND**
48	-	/	/	-	19.41	31.49	+	+	+	-	+	+	+	**PD**
26	+	-	+	+	19.96	30.37	+	+	+	-	+	+	+	**PA**
29	-	/	/	-	18.63	30.78	+	+	+	-	+	+	+	**PD**
31	+	-	+	+	N/A	30.82	-	-	-	/	/	-	-	**ND**
34	+	-	+	+	18.84	30.64	+	+	+	-	+	+	+	**PA**
36	-	/	/	-	18.63	31.31	+	+	+	-	+	+	+	**PD**
40	+	-	+	+	19.01	31.10	+	+	+	-	+	+	+	**PA**
43	+	-	+	+	33.15	30.62	+	+	+	-	+	+	+	**PA**
45	+	-	+	+	19.40	30.78	+	+	+	-	+	+	+	**PA**

*Standardized **report** -Qualitative methods*
Summary Report
[2018LR80 - **iQ-Check EB** - 02 September 2025]

Laboratory **D** CFX96 DW
Lactic acid bacteria: 0 CFU/g

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	-	/	/	-	N/A	30.61	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	30.68	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	30.43	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	30.57	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	30.3	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	30.43	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.25	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	31.41	-	-	-	/	/	-	-	**NA**
3	+	-	+	+	31.33	30.52	+	+	+	-	+	+	+	**PA**
8	+	-	+	+	35.34	30.35	+	+	+	-	+	+	+	**PA**
11	+	-	+	+	24.31	30.31	+	+	+	-	+	+	+	**PA**
12	+	-	+	+	17.32	31.62	+	+	+	-	+	+	+	**PA**
15	-	/	/	-	18.14	30.98	+	+	+	-	+	+	+	**PD**
17	-	/	/	-	20.04	31.14	+	+	+	-	+	+	+	**PD**
20	+	-	+	+	N/A	30.43	-	-	-	/	/	-	-	**ND**
22	+	-	+	+	17.14	33.16	+	+	+	-	+	+	+	**PA**
1	+	-	+	+	17.81	31.37	+	+	+	-	+	+	+	**PA**
4	+	-	+	+	16.91	35.13	+	+	+	-	+	+	+	**PA**
7	+	-	+	+	17.52	32.55	+	+	+	-	+	+	+	**PA**
10	+	-	+	+	17.88	31.54	+	+	+	-	+	+	+	**PA**
13	+	-	+	+	17.9	31.31	+	+	+	-	+	+	+	**PA**
16	+	-	+	+	17.32	31.34	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	16.98	31.64	+	+	+	-	+	+	+	**PA**
23	+	-	+	+	17.28	31.56	+	+	+	-	+	+	+	**PA**

Laboratory **E**
Lactic acid bacteria: 8.2 x 10^6 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check Enterobacteriaceae									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	-	/	/	-	N/A	31.19	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	30.64	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	31.14	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	31.08	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	30.71	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	31.09	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.92	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	30.94	-	-	-	/	/	-	-	**NA**
3	+	+	+	+	N/A	30.69	-	-	-	/	/	-	-	**ND**
8	+	+	+	+	N/A	31.63	-	-	-	/	/	-	-	**ND**
11	+	+	+	+	17.79	32.26	+	+	+	-	+	+	+	**PA**
12	+	+	+	+	N/A	30.76	-	-	-	/	/	-	-	**ND**
15	+	+	+	+	18.04	31.77	+	+	+	-	+	+	+	**PA**
17	+	+	+	+	21.6	32.35	+	+	+	-	+	+	+	**PA**
20	+	+	+	+	27.2	31.16	+	+	+	-	+	+	+	**PA**
22	+	+	+	+	N/A	30.95	-	-	-	/	/	-	-	**ND**
1	+	+	+	+	17.08	32.02	+	+	+	-	+	+	+	**PA**
4	-	/	/	-	29.09	30.68	+	+	+	-	+	+	+	**PD**
7	+	+	+	+	17.57	31.69	+	+	+	-	+	+	+	**PA**
10	+	+	+	+	N/A	30.76	-	-	-	/	/	-	-	**ND**
13	+	+	+	+	27.68	30.41	+	+	+	-	+	+	+	**PA**
16	+	+	+	+	-/35.69/34.19	31.51/30.77/30.65	-/+/+	+	+	-	+	+	-	**$ND_{FN(alt)}$**
18	+	+	+	+	29.82	30.75	+	+	+	-	+	+	+	**PA**
23	+	+	+	+	28.53	30.77	+	+	+	-	+	+	+	**PA**

Laboratory **F1**
Lactic acid bacteria: CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	-	/	/	-	N/A	31.3	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	31.22	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	31.26	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	31.16	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	31.28	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	31.24	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	31.25	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	31.34	-	-	-	/	/	-	-	**NA**
3	-	/	/	-	18.02	31.99	+	+	+	-	+	+	+	**PD**
8	-	/	/	-	18.41	32.04	+	+	+	-	+	+	+	**PD**
11	+	-	+	+	18.5	32.91	+	+	+	-	+	+	+	**PA**
12	-	/	/	-	18.26	31.77	+	+	+	-	+	+	+	**PD**
15	+	-	+	+	18.69	32.04	+	+	+	-	+	+	+	**PA**
17	+	-	+	+	17.59	32.58	+	+	+	-	+	+	+	**PA**
20	-	/	/	-	N/A	31.39	-	-	-	/	/	-	-	**NA**
22	+	-	+	+	18.11	32.53	+	+	+	-	+	+	+	**PA**
1	+	-	+	+	17.82	32.18	+	+	+	-	+	+	+	**PA**
4	+	-	+	+	17.51	32.56	+	+	+	-	+	+	+	**PA**
7	+	-	+	+	19.16	32.41	+	+	+	-	+	+	+	**PA**
10	-	/	/	-	19.04	31.84	+	+	+	-	+	+	+	**PD**
13	+	-	+	+	18.59	31.98	+	+	+	-	+	+	+	**PA**
16	+	-	+	+	18.85	32.26	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	21.25	31.5	+	+	+	-	+	+	+	**PA**
23	-	/	/	-	N/A	31.47	-	-	-	/	/	-	-	**NA**

Laboratory **F2**
Lactic acid bacteria: CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check Enterobacteriaceae									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
25	-	/	/	-	N/A	31.39	-	-	-	/	/	-	-	**NA**
28	-	/	/	-	N/A	31.4	-	-	-	/	/	-	-	**NA**
30	-	/	/	-	N/A	31.42	-	-	-	/	/	-	-	**NA**
35	-	/	/	-	N/A	31.43	-	-	-	/	/	-	-	**NA**
38	-	/	/	-	N/A	31.35	-	-	-	/	/	-	-	**NA**
41	-	/	/	-	N/A	31.25	-	-	-	/	/	-	-	**NA**
44	-	/	/	-	N/A	31.31	-	-	-	/	/	-	-	**NA**
47	-	/	/	-	N/A	31.57	-	-	-	/	/	-	-	**NA**
27	+	-	+	+	17.6	32.05	+	+	+	-	+	+	+	**PA**
32	+	-	+	+	17.86	32.64	+	+	+	-	+	+	+	**PA**
33	+	-	+	+	26.58	31.08	+	+	+	-	+	+	+	**PA**
37	+	-	+	+	22.6	31.25	+	+	+	-	+	+	+	**PA**
39	-	/	/	-	18.25	32.38	+	+	+	-	+	+	+	**PD**
42	-	/	/	-	N/A	31.43	-	-	-	/	/	-	-	**NA**
46	-	/	/	-	17.81	32.9	+	+	+	-	+	+	+	**PD**
48	-	/	/	-	17.83	31.95	+	+	+	-	+	+	+	**PD**
26	+	-	+	+	25.65	31.17	+	+	+	-	+	+	+	**PA**
29	+	-	+	+	18.26	32.39	+	+	+	-	+	+	+	**PA**
31	+	-	+	+	18.37	32.37	+	+	+	-	+	+	+	**PA**
34	-	/	/	-	18.03	32.4	+	+	+	-	+	+	+	**PD**
36	+	-	+	+	17.51	32.17	+	+	+	-	+	+	+	**PA**
40	+	-	+	+	18.2	32.33	+	+	+	-	+	+	+	**PA**
43	+	-	+	+	17.95	32.43	+	+	+	-	+	+	+	**PA**
45	-	/	/	-	17.75	32.48	+	+	+	-	+	+	+	**PD**

Laboratory **G1**
Lactic acid bacteria: 1.9 x 10^6 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	-	/	/	-	N/A	30.46	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	30.7	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	30.69	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	31.44	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	31.28	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	30.66	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.58	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	30.56	-	-	-	/	/	-	-	**NA**
3	+	+	+	+	18.84	31.35	+	+	+	-	+	+	+	**PA**
8	-	/	/	-	18.87	30.87	+	+	+	-	+	+	+	**PD**
11	-	/	/	-	18.85	36.24	+	+	+	-	+	+	+	**PD**
12	+	+	+	+	18.51	N/A	+	+	+	-	+	+	+	**PA**
15	+	+	+	+	19.16	33.79	+	+	+	-	+	+	+	**PA**
17	-	/	/	-	18.64	35.23	+	+	+	-	+	+	+	**PD**
20	+	+	+	+	N/A	30.74	-	-	-	/	/	-	-	**ND**
22	-	/	/	-	22.33	30.51	+	+	+	-	+	+	+	**PD**
1	+	+	+	+	22.45	30.3	+	+	+	-	+	+	+	**PA**
4	+	+	+	+	18.76	31.78	+	+	+	-	+	+	+	**PA**
7	+	+	+	+	20.05	30.69	+	+	+	-	+	+	+	**PA**
10	+	+	+	+	23.95	30.84	+	+	+	-	+	+	+	**PA**
13	+	+	+	+	18.45	N/A	+	+	+	-	+	+	+	**PA**
16	+	+	+	+	18.65	31.12	+	+	+	-	+	+	+	**PA**
18	+	+	+	+	18.65	33.56	+	+	+	-	+	+	+	**PA**
23	+	+	+	+	21.27	30.67	+	+	+	-	+	+	+	**PA**

Laboratory **G2**
Lactic acid bacteria: 2.2 x 10^6 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check Enterobacteriaceae									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
25	-	/	/	-	N/A	30.93	-	-	-	/	/	-	-	**NA**
28	-	/	/	-	N/A	30.85	-	-	-	/	/	-	-	**NA**
30	-	/	/	-	N/A	30.81	-	-	-	/	/	-	-	**NA**
35	-	/	/	-	N/A	31.01	-	-	-	/	/	-	-	**NA**
38	-	/	/	-	N/A	30.93	-	-	-	/	/	-	-	**NA**
41	-	/	/	-	N/A	30.86	-	-	-	/	/	-	-	**NA**
44	-	/	/	-	N/A	30.75	-	-	-	/	/	-	-	**NA**
47	-	/	/	-	N/A	30.95	-	-	-	/	/	-	-	**NA**
27	+	+	+	+	N/A	32.12	-	-	-	/	/	-	-	**ND**
32	-	/	/	-	N/A	30.7	-	-	-	/	/	-	-	**NA**
33	+	+	+	+	N/A	30.33	-	-	-	/	/	-	-	**ND**
37	+	+	+	+	N/A	30.63	-	-	-	/	/	-	-	**ND**
39	+	+	+	+	17.47	31.3	+	+	+	-	+	+	+	**PA**
42	-	/	/	-	N/A	31.03	-	-	-	/	/	-	-	**NA**
46	+	+	+	+	N/A	30.84	-	-	-	/	/	-	-	**ND**
48	-	/	/	-	21.86	30.15	+	+	+	-	+	+	+	**PD**
26	-	/	/	-	22.26	30.25	+	+	+	-	+	+	+	**PD**
29	+	+	+	+	N/A	30.7	-	-	-	/	/	-	-	**ND**
31	-	/	/	-	18.46	30.72	+	+	+	-	+	+	+	**PD**
34	-	/	/	-	17.58	32.45	+	+	+	-	+	+	+	**PD**
36	+	+	+	+	18.13	31.67	+	+	+	-	+	+	+	**PA**
40	-	/	/	-	18.43	31.39	+	+	+	-	+	+	+	**PD**
43	-	/	/	-	22.5	30.3	+	+	+	-	+	+	+	**PD**
45	+	+	+	+	20.34	30.59	+	+	+	-	+	+	+	**PA**

Laboratory **H1**
Lactic acid bacteria: 1.6 x 10^5 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	-	/	/	-	N/A	30.68	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	30.7	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	30.53	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	30.89	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	30.48	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	30.59	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.45	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	28.52/-*/-*	30.5/31.01/30.85	+/-/-	-	-	/	/	-	-	**$PD_{FP(alt)}$**
3	+	-	+	+	26.78	30.36	+	+	+	-	+	+	+	**PA**
8	+	-	+	+	N/A	30.7	-	-	-	/	/	-	-	**ND**
11	+	-	+	+	N/A	30.62	-	-	-	/	/	-	-	**ND**
12	+	-	+	+	25.69	60.28	+	+	+	-	+	+	+	**PA**
15	-	/	/	-	25.99	30.25	+	+	+	-	+	+	+	**PD**
17	+	-	+	+	25.21	30.18	+	+	+	-	+	+	+	**PA**
20	+	-	+	+	24.4	30.16	+	+	+	-	+	+	+	**PA**
22	+	-	+	+	N/A	30.46	-	-	-	/	/	-	-	**ND**
1	-	/	/	-	26.68	30.54	+	+	+	-	+	+	+	**PD**
4	-	/	/	-	25.02	30.21	+	+	+	-	+	+	+	**PD**
7	+	-	+	+	24.53	30.15	+	+	+	-	+	+	+	**PA**
10	-	/	/	-	26.65	30.33	+	+	+	-	+	+	+	**PD**
13	+	-	+	+	25.57	30.29	+	+	+	-	+	+	+	**PA**
16	+	-	+	+	25.57	30.2	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	28.21	30.46	+	+	+	-	+	+	+	**PA**
23	+	-	+	+	28.26	30.39	+	+	+	-	+	+	+	**PA**

Laboratory **H2**
Lactic acid bacteria: 1.19 x 10^5 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG					
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)			
25	-	/	/	-	N/A	30.87	-	-	-	/	/	-	-	**NA**
28	-	/	/	-	N/A	30.6	-	-	-	/	/	-	-	**NA**
30	-	/	/	-	N/A	30.56	-	-	-	/	/	-	-	**NA**
35	-	/	/	-	N/A	30.97	-	-	-	/	/	-	-	**NA**
38	-	/	/	-	N/A	30.71	-	-	-	/	/	-	-	**NA**
41	-	/	/	-	N/A	30.71	-	-	-	/	/	-	-	**NA**
44	-	/	/	-	N/A	30.63	-	-	-	/	/	-	-	**NA**
47	-	/	/	-	N/A	31.12	-	-	-	/	/	-	-	**NA**
27	+	-	+	+	25.88	30.32	+	+	+	-	+	+	+	**PA**
32	+	-	+	+	N/A	30.76	-	-	-	/	/	-	-	**ND**
33	-	/	/	-	26.36	30.34	+	+	+	-	+	+	+	**PD**
37	+	-	+	+	29.98	30.54	+	+	+	-	+	+	+	**PA**
39	+	-	+	+	25.89	30.37	+	+	+	-	+	+	+	**PA**
42	+	-	+	+	25.96	30.27	+	+	+	-	+	+	+	**PA**
46	-	/	/	-	N/A	30.64	-	-	-	/	/	-	-	**NA**
48	+	-	+	+	25.87	30.32	+	+	+	-	+	+	+	**PA**
26	+	-	+	+	N/A	30.68	-	-	-	/	/	-	-	**ND**
29	+	-	+	+	24.15	30.23	+	+	+	-	+	+	+	**PA**
31	+	-	+	+	25.66	30.38	+	+	+	-	+	+	+	**PA**
34	+	-	+	+	25.33	30.3	+	+	+	-	+	+	+	**PA**
36	+	-	+	+	35	30.64	+	+	+	-	+	+	+	**PA**
40	+	-	+	+	25.88	30.31	+	+	+	-	+	+	+	**PA**
43	-	/	/	-	N/A	30.63	-	-	-	/	/	-	-	**NA**
45	+	-	+	+	25.78	30.31	+	+	+	-	+	+	+	**PA**

Laboratory **I**
Lactic acid bacteria: 1.1 x 10^6 CFU/g CFX96 DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	+ *	-	+	unknown	26.8	32.12	+	-	-	/	/	-	-	**unknown**
5	-	/	/	-	N/A	32.14	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	27.89	32	+	-	-	/	/	-	-	**$PD_{FP(alt)}$**
9	-	/	/	-	30.02	31.28	+	-	-	/	/	-	-	**$PD_{FP(alt)}$**
14	-	/	/	-	N/A	32.09	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	31.57 and N/A **	30.99		-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	31.01	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	30.73	-	-	-	/	/	-	-	**NA**
3	+ *	-	+	unknown	17	N/A	+	+	+	-	+	+	+	**unknown**
8	+	-	+	+	16.75	36.31	+	+	+	-	+	+	+	**PA**
11	+	-	+	+	16.31	N/A	+	+	+	-	+	+	+	**PA**
12	+	-	+	+	16.77	N/A	+	+	+	-	+	+	+	**PA**
15	-	/	/	-	16.83	36.16	+	+	+	-	+	+	+	**PD**
17	+	-	+	+	16.07	33.03	+	+	+	-	+	+	+	**PA**
20	+	-	+	+	N/A/N/A	30.83	-	+ not confirmed	+ / - ***	-	-	-	-	**ND**
22	-			-	N/A	31.08	-	-	-	/	/	-	-	**NA**
1	+	-	+	+	16.46	N/A	+	+	+	-	+	+	+	**PA**
4	+	-	+	+	16.83	N/A	+	+	+	-	+	+	+	**PA**
7	+	-	+	+	16.86	N/A	+	+	+	-	+	+	+	**PA**
10	+	-	+	+	17.18	N/A	+	+	+	-	+	+	+	**PA**
13	-	/	/	-	16.86	N/A	+	+	+	-	+	+	+	**PD**
16	+	-	+	+	16.87	34.92	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	16.03	32.38	+	+	+	-	+	+	+	**PA**
23	-			-	N/A	31.08	-	-	-	/	/	-	-	**NA**

* Samples mixed during the study

MICROVAL® NEN

Laboratory **J**
Lactic acid bacteria: 4.6 x 10^5 CFX Opus DW

N°Sample	Reference method: ISO 21528-1				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
	VRBG	Confirmation		Reference final result	PCR result		PCR final result	Confirmation				Confirmation final result	Final result	
					DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG	Oxydase test	OF media (glucose fermentation test)			
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)		*Enterobacteriaceae* Cq	IC Cq		Typical colonies (+/-)	Typical colonies (+/-)					
2	-	/	/	-	N/A	30.88	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	21.86/-/-	31.39/30.73/21.47	+/-/-	-	-	/	/	-	-	**$PD_{FP(alt)}$**
6	-	/	/	-	22.31/-/-	31.31/30.40/30.55	+/-/-	-	-	/	/	-	-	**$PD_{FP(alt)}$**
9	-	/	/	-	N/A	31.08	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	30.88	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	31.08	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.75	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	36.66/20.88/30.25	31.14/20.16/35.53	+/+/+	-	-	/	/	-	-	**$PD_{FP(alt)}$**
3	+	-	+	+	N/A	31.08	-	-	-	/	/	-	-	**ND**
8	+	-	+	+	25.39	31.07	+	+	+	-	+	+	+	**PA**
11	+	-	+	+	29.92	31.01	+	+	+	-	+	+	+	**PA**
12	-	/	/	-	27.48	30.97	+	+	+	-	+	+	+	**PD**
15	+	-	+	+	31.76	30.76	+	+	+	-	+	+	+	**PA**
17	-	/	/	-	N/A	31.07	-	-	-	/	/	-	-	**NA**
20	+	-	+	+	N/A	30.88	-	-	-	/	/	-	-	**ND**
22	+	-	+	+	19.06	31.8	+	+	+	-	+	+	+	**PA**
1	+	-	+	+	N/A/20.34/24.94	31.31/32.33/30.41	-	+	+	-	+	+	-	**$ND_{FN(alt)}$**
4	-	/	/	-	21.51	32.17	+	+	+	-	+	+	+	**PD**
7	+	-	+	+	N/A/20.31/24.38	31.05/32.08/30.25	-	+	+	-	+	+	-	**$ND_{FN(alt)}$**
10	+	-	+	+	N/A	31.22	-	-	-	/	/	-	-	**ND**
13	+	-	+	+	N/A/30.75/28.74	31.33/30.59/30.85	-	+	+	-	+	+	-	**$ND_{FN(alt)}$**
16	-	/	/	-	29.51	30.72	+	+	+	-	+	+	+	**PD**
18	+	-	+	+	30.28	30.84	+	+	+	-	+	+	+	**PA**
23	+	-	+	+	34.79	31.07	+	+	+	-	+	+	+	**PA**

Laboratory **K (ADRIA)**
Lactic acid bacteria: 6.8 x 10^6 CFU/g

N°Sample	Reference method: ISO 21528-1♦				Alternative method: iQ-Check *Enterobacteriaceae*									Agreement Ref/Alt
		Confirmation			PCR result			Confirmation						
	VRBG				DNA extraction Easy I lysis with FDRS			RAPID'*Enterobacteriaceae*	VRBG					
	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)	Reference final result	*Enterobacteriaceae* Cq	IC Cq	PCR final result	Typical colonies (+/-)	Typical colonies (+/-)	Oxydase test	OF media (glucose fermentation test)	Confirmation final result	Final result	
2	-	/	/	-	N/A	30.41	-	-	-	/	/	-	-	**NA**
5	-	/	/	-	N/A	31.03	-	-	-	/	/	-	-	**NA**
6	-	/	/	-	N/A	31	-	-	-	/	/	-	-	**NA**
9	-	/	/	-	N/A	31.35	-	-	-	/	/	-	-	**NA**
14	-	/	/	-	N/A	30.94	-	-	-	/	/	-	-	**NA**
19	-	/	/	-	N/A	30.96	-	-	-	/	/	-	-	**NA**
21	-	/	/	-	N/A	30.82	-	-	-	/	/	-	-	**NA**
24	-	/	/	-	N/A	31.53	-	-	-	/	/	-	-	**NA**
3	-	/	/	-	18.07	34.66	+	+	+	-	+	+	+	**PD**
8	+	-	+	+	18	31.61	+	+	+	-	+	+	+	**PA**
11	-	/	/	-	20.32	N/A	+	+	+	-	+	+	+	**PD**
12	-	/	/	-	N/A	31.1	-	-	-	/	/	-	-	**NA**
15	-	/	/	-	19.55	35.9	+	+	+	-	+	+	+	**PD**
17	+	-	+	+	33.44	30.86	+	+	+	-	+	+	+	**PA**
20	+	-	+	+	25.33	30.86	+	+	+	-	+	+	+	**PA**
22	+	-	+	+	17.75	N/A	+	+	+	-	+	+	+	**PA**
1	+	-	+	+	18.39	31.92	+	+	+	-	+	+	+	**PA**
4	+	-	+	+	37.38	30.47	+	+	+	-	+	+	+	**PA**
7	+	-	+	+	17.99	31.37	+	+	+	-	+	+	+	**PA**
10	+	-	+	+	17.93	33.74	+	+	+	-	+	+	+	**PA**
13	-	/	/	-	17.66	N/A	+	+	+	-	+	+	+	**PD**
16	+	-	+	+	17.72	N/A	+	+	+	-	+	+	+	**PA**
18	+	-	+	+	18.35	N/A	+	+	+	-	+	+	+	**PA**
23	+	-	+	+	19.08	32.46	+	+	+	-	+	+	+	**PA**

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