NF VALIDATION 16140

AFNOR Certification Validation of the method

ADIAFOOD *Cronobacter* spp

Protocol for milk powders, infant formulas and dehydrated complements
Protocol for matrices used in the composition of milk powders and infant formulas
Protocol for environmental samples from milk powders and infant formulas industries

SUMMARY REPORT– DECEMBER 2013

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1. Introduction

1.1. Validation referential

The aim of this validation study is to evaluate the performance of the alternative method against the reference method ISO/TS 22964 (2006) . It consists in a preliminary study and a collaborative study.

1.2. Alternative method

The ADIAFOOD system is based on Real-Time Polymerase Chain Reaction (PCR) technology. The system provides rapid detection by specifically identifying the DNA sequence of pathogens in a series of sequential steps that include sample preparation and enrichment, DNA extraction, and pathogen detection.
The thermocycler used for the validation was a MX 3005P from Stratagene, used with the Sentinel software number 2.0.0 – R 9 3.

The protocol of the method is showed in appendix 1.

During the detection step, the target pathogen DNA is amplified and detected using specific primers and molecular beacons, the key components of the AES CHEMUNEX technology. Molecular beacons probes consist in a unique sequence probe that allows the identification of the pathogen with a high level of specificity. Once bound to their target, the molecular beacons emit a fluorescent signal that is proportional to the amount of amplified pathogenic DNA. In the absence of target bacteria in food samples, no fluorescent signal is detected. Results are recorded and analyzed automatically with AES CHEMUNEX' proprietary Sentinel software.

1.3. Scope of application

The alternative method was tested for three categories:

- milk powders, infant formulas and dehydrated complements
- matrices used in the composition of milk powders and infant formulas
- environmental samples from milk powders and infant formulas industries

1.4. Reference method(*)

The standard ISO/TS 22964 (2006) : Milk and dairy products – Detection of *Enterobacter sakazakii* was applied. The protocol of this method is shown in appendix 2.

2. Comparative study

The following characteristics are studied during the preliminary study:

- Relative accuracy (AC), relative specificity (SP) and relative sensitivity (SE)
- Relative detection level of the alternative method and the reference method
- Selectivity of the alternative method
- Practicability of the alternative method

2.1. Relative accuracy, relative specificity, relative sensitivity

The relative accuracy is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples.
The relative specificity is the ability of the alternative method to not detect the target microorganism when it is not detected by the reference method.

The relative sensitivity is the ability of the alternative method to detect the analyte when it is detected by the reference method.

The objective of this study is to evaluate the performance of both methods on contaminated and non-contaminated samples.

2.1.1. Number and nature of samples

A number of 235 samples was analysed. Types of products are indicated in table 1.

Category	Type	Number of positive*	Number of negative	Total
Milk powders, infant formulas and dehydrated complements	Milk powders / Infant formulas powders with or without probiotics	21	25	46
	Liquid infant formulas	5	3	8
	Dehydrated complements	4	4	8
	Total	**30**	**32**	**62**
Matrices used in the composition of milk powders and infant formulas	Proteins	16	15	31
	Lactose/lactoserum/various	8	9	17
	Soy milk/Others	8	8	16
	Total	**32**	**32**	**64**
Environmental samples from milk powders and infant formulas industries	Surface swabs	31	33	64
	Dusts and residues	10	15	25
	Process waters	10	10	20
	Total	**51**	**58**	**109**
Total		**113**	**122**	**235**

Table 1 : nature and number of samples analyzed (*positive results by either method)

2.1.2. Artificial contamination of samples

Five naturally contaminated samples were analyzed. Spikings were realized on the 108 other positive samples.

For spiking, several strains were stressed using different treatments and the stress intensity was evaluated (logarithmic difference between enumeration on non selective agar –TSA- and selective agar –ESIA-, cf. appendix 3).

2.1.3. Results

Each sample was analysed once by the alternative method and once by the reference method. Table 2 presents paired results of both methods. Raw results in appendix 4.

Category	Response	Reference method (*) positive (R+)	Reference method (*) negative (R-)
Milk powders, infant formulas and dehydrated complements	**Alternative method positive (A+)**	PA=26	PD=3
	Alternative method negative (A-)	ND=1 with 0 PPND	NA=32 with 1 PPNA
Matrices used in the composition of milk powders and infant formulas	**Alternative method positive (A+)**	PA=31	PD=0
	Alternative method negative (A-)	ND=1 with 0 PPND	NA=32 with 0 PPNA
Surface swabs at 8h and 24h	**Alternative method positive (A+)**	PA=30	PD=1
	Alternative method negative (A-)	ND=0 with 0 PPND	NA=33 with 1 PPNA
Dusts and process waters	**Alternative method positive (A+)**	PA=25	PD=0
	Alternative method negative (A-)	ND= 0 with 0 PPND	NA=20 with 0 PPNA
All categories	**Alternative method positive (A+)**	PA=112	PD=4
	Alternative method negative (A-)	ND=2 dont 0 PPND	NA=117 with 2 PPNA

Table 2: results of relative accuracy for both methods (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, PP: presumed positive before confirmation, A+: confirmed positive, A-: negative immediately and negative after confirmation when presumed positive)

2.1.4. Calculation of relative accuracy (AC), relative specificity (SP) and relative sensitivity (SE)

For all products categories, these results permit to calculate the relative accuracy, relative specificity and relative sensitivity according to NF EN ISO standard. Results are indicated in table 3.

Category	PA	NA	ND	PD	N	Relative accuracy **AC** [(PA+NA)/N]	**N+** PA+ND	Relative sensitivity **SE** [PA/N+]	**N-** NA+PD	Relative specificity **SP** [NA/N-]
Milk powders, infant formulas and dehydrated complements	26	32	1	3	62	93,5%	27	96,3%	35	91,4%
Matrices used in the composition of milk powders and infant formulas	31	32	1	0	64	98,4%	32	96,9%	32	100,0%
Surface swabs at 8h and 24h	30	33	0	1	64	98,4%	30	100,0%	34	97,1%
Dusts and process waters	25	20	0	0	45	100%	25	100%	20	100%
All categories	112	117	2	4	235	97,4%	114	98,2%	121	96.7%

Table 3: relative accuracy, relative specificity and relative sensitivity of alternative method (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation, AC = (PA+NA)/N x 100%, SE = PA/N+ x 100%, SP = NA/N- x 100%, N+ = PA+ND and N- = NA+PD)

Criteria values in percent are shown in table 4.

	Alternative method
Relative accuracy	97,4%
Relative sensitivity	98,2%
Relative specificity	96,7%

Table 4: AC, SE and SP in percent for alternative method

Sensitivity of both methods was recalculated considering all confirmed positive (including alternative method positive deviations). Results are shown in table 5.

	Alternative method (PA+PD)/(PA+PD+ND)	**Reference method** (PA+ND)/(PA+PD+ND)
Sensitivity	98,3%	96,6%

Table 5: sensitivity of both methods including all confirmed positive

2.1.5. Analysis of discordant results

Discordant results are examined according to annex F of NF EN ISO 16140 standard, with Y as the number of discordant results and m as the smallest of the two values of PD and ND. Statistical analysis is shown in table 6.

	Alternative method
Y = PD + ND	Y = 4 + 2 = 6
m	2
M	0
Conclusion	m > M, the methods are identical at $\alpha < 0,05$

Table 6 : statistical analysis of discordant results

The two methods are considered as equivalent.

- Positive deviations : AC125, AC133, AC 217, AC246

A confirmed positive result is obtained by the alternative method whereas a negative result is obtained by the reference method.
Any colony was found on the ESIA Petri dishes of the reference method. It could be due to a lack of sensitivity of the reference method.

- Negative deviations : AC264, AC182

A positive result is obtained by the reference method whereas a negative result is observed after amplification and the confirmation protocol shows the presence of *Cronobacter* in the broth.
This absence of detection could be due to a lack of sensitivity of the alternative method in a broth with a very low concentration in target bacteria. The volume used for the amplification (10 µL) being very inferior to the volume used for the confirmation (500 µL), it's possible that no cell of Cronobacter was present in the volume used for the detection with the alternative method.

2.1.6. Study of the conservation of the broths

A stability study of the enriched broths stored at 5±3°C for 72 hours was performed on all positive and discordant samples. The broths were re-analyzed and confirmed after storage (results in appendix 5).
The analysis result of only one sample was different after storage (AC182). It was a false negative and it became positive.

This result did not modify the conclusion of the statistical analysis for the conservation of the broths.

2.2. Relative detection level

The objective of this study is to determine the level of contamination for which less than 50% of the responses obtained are positive and that for which more than 50% of the responses obtained are positive.

2.2.1. Matrices

A couple "matrix-strain" was studied in parallel with the reference method and the alternative method for each category. The total viable count of each matrix was enumerated. Characteristics of the strain and the matrix are shown in table 6.

Matrix	Strain	Code	Strain origin
Infant formula powder	*Cronobacter sakazakii*	ENTB.3.1	Milk powder
Soy milk	*Cronobacter muytjensi*	ENTB.3.21	CIP 103581
Swabs	*Cronobacter malonaticus*	ENTB.3.38	Workshop environment

Table 7 : couples « strain-matrix » for the relative detection levels

2.2.2. Spiking protocol

Six levels of contamination were tested including the negative control.
Six replicates for each level of contamination were inoculated and analysed by the reference method and the alternative method.
As the two methods have no common step, 12 test portions of 50 g were prepared for each level of contamination and individually inoculated with a calibrated bacterial suspension. Bacterial suspension of about 10 cells per mL was prepared. From this initial suspension, volumes of 0.9 mL, 0.3 mL and 0.1 mL were used to spike 50 g of sample respectively for the 3 first levels. In parallel, the initial suspension was diluted ratio ½ and ¼ in order to inoculate the lower levels of contamination with 0.1 mL.

For all the levels of contamination, homogeneity of the inoculums was checked by enumeration on 30 TSA Petri dishes. Then, the confidence interval was determined according to Poisson law.

2.2.3. Results

Tables 8 and 9 present the relative detection level for each method. Raw results in appendix 5.

		RLOD according to Spearman-Kärber (50 g/mL)	
Strain	**Matrix**	**Reference method(*)**	**Alternative method**
ENTB.3.1	Poudre de lait	0,775 [0,521 ; 1,138]	0,775 [0,521 ; 1,138]
ENTB.3.21	Lait de soja	1,131 [0,770 ; 1,661]	1,025 [0,698 ; 1,506]
ENTB.3.38	Ecouvillon	0,822 [0,612 ; 1,097]	0,968 [0,628 ; 1,492]

Table 8 : relative level of detection (3 significant digits)

		RLOD according to Spearman-Kärber (50 g/mL)	
Strain	**Matrix**	**Reference method(*)**	**Alternative method**
ENTB.3.1	Poudre de lait	0,8 [0,5 ; 1,1]	0,8 [0,5 ; 1,1]
ENTB.3.21	Lait de soja	1,1 [0,8 ; 1,7]	1,0 [0,7 ; 1,5]
ENTB.3.38	Ecouvillon	0,8 [0,6 ; 1,1]	1,0 [0,6 ;1,5]

Table 9 : niveau de détection relatif (1 significant digit)

The alternative and the reference method show similar detection levels. The detection limit obtained is comprised between 0.5 and 1.5 CFU in 50 g for the alternative method and between 0.5 and 1.7 CFU in 50 g for the reference method.

2.3. Inclusivity / exclusivity (selectivity)

The objective of this study is to test:

-the inclusivity: the detection of the target microorganism from a wide range of strains,

-the exclusivity: the lack of interference from a relevant range of non-target microorganisms.

According to the requirements of NF EN ISO 16140, 50 strains of *Cronobacter* spp and 30 non-target strains were tested.

2.3.1. Test protocols

- **Inclusivity**

Each target strain was cultivated twice before inoculation in the broth of the alternative method (about 1 to 100 CFU/450 mL). The complete protocol of alternative method was applied with the minimum time of incubation.

- **Exclusivity**

Each non-target strain was cultivated twice before inoculation in growth medium (Tryticase Soy Broth) with a level of contamination expected to occur in the food matrices (about 10^5 CFU/mL). After 24 hours of incubation, the ADIAFOOD test was performed.

In cases where the target strains or non-target strains results were unexpected to interpret by the alternative method, the analysis was conducted once again in parallel with the alternative method and the reference method (complete protocol).

2.3.2. Results

Raw results in appendix 6.

The 50 *Cronobacter* strains were detected by the alternative method.

No non target strain was detected by the alternative method.

2.3.3. Conclusion

The selectivity of the method is satisfactory.

2.4. Practicability

The practicability was evaluated according to the 13 criteria defined by AFNOR Technical Committee.

1- Mode of packaging of test components

2- Volume of reagents

COMPONENT	DESCRIPTION	Format or volume
Detection microplates or barrettes	Microplates / barrettes for the real-time PCR detection	ADIF1316 : 4 microplates - 88 tests ADIF6116 : 12 barrettes - 8 tests
Control barrettes	Control barretttes specific of the pathogen	ADIF8116 : 8 control barrettes
DT-1	solution used for the reconstitution of lyophilised DT-2	2.2 mL
DT-2	Lyophilised compound for the detection solution	/
EX-1	solution used for the reconstitution of lyophilised EX-2	10 mL
EX-2	Lyophilised compound for the extraction solution	/
Optical quality PCR corks	Flat corks	/
User guide	Protocol	/

3- Storage conditions of components and shelf-life of unopened products (expiration of not opened products)

4- Modalities after first use

The ADIAFOOD detection kits must be stored at 4°C (from 2 to 8°C) in their original packaging.

After extraction, microplates can be stored at 4°C (from 2 to 8°C) for a maximum of 24 hours or at -20°C for several months. Do not remove the domed caps before storing.
The shelf life of the reconstituted solutions EX-2 and DT-2 is 14 days at 4°C (from 2 to 8°C) in their original vials, sealed by a Parafilm.

Never exchange the components of different lots or from other sources.

5- Equipment and specific local requirements

Equipment

- Real-time PCR thermocycler validated by AES : STRATAGENE MX3005P or MX3000P
- Centrifuge
- Capping tool
- Barrettes supports
- Two multi-channel pipettes
- Two single channel pipettes
- PCR encloser
- *Stomacher* (homogenizer)
- Incubators
- Dilutor
- Bunsen burner
- Serological pipette pump
- Stomacher bag holder
- Refrigerator 4°C (2 to 8°C)
- Colour printer
- Pipettes supports
- Tubes racks

Consumables

- ADIAFOOD extraction kit
- Aerosol barrier tips for pipetors
- Sterile microcentrifuge cap tubes
- Free powder gloves
- Denatured alcool and bleach
- Disposable basins for multi-channel pipettes
- Filter stomacher bags
- Disposable serological pipettes
- Parafilm
- Media required : peptone water, novobiocin supplement, ESIA agar media or ChromID Sakazakii agar media

6- Reagents ready to use or for reconstitution
DT-2 and EX-2 components must be reconstituted in DT-1 and EX-1.

7- Training period for operator with no experience with the method
1 day is required for technicians with microbiology knowledge.

8- Handling time and flexibility of the method in relation to the number of samples

Steps– Manipulation time – Negative samples	**Time (minutes)**			
	Alternative method		**Reference method**	
	1 analysis	**20 analyses**	**1 analysis**	**20 analyses**
Suspension preparation	3	15	3	15
Amplification	3	10	/	/
PCR reading	1	3	/	/
mLST inoculation	/	/	1	10
Selective agar media isolation	/	/	0,5	5
Petri dishes reading	/	/	1	10
Total	**7**	**28**	**5,5**	**40**

Steps– Manipulation time – positive samples	Time (minutes)			
	Alternative method		Alternative method	
	1 analysis	1 analysis	1 analysis	1 analysis
Suspension preparation	3	15	3	15
Amplification	3	10	/	/
PCR reading	1	3	/	/
mLST inoculation	1	10	1	10
Selective agar media isolation	0,5	5	0,5	5
Petri dishes reading	1	10	1	10
Purification on TSA	/	/	0,5	5
Biochemical confirmation	/	/	2	15
Reading	/	/	2	30
Total	**8,5**	**53**	**10**	**90**

9- Time-to-result

Categories: milk powders / infant formulas / dehydrated complements / matrices used in the composition of milk powders and infant formulas / environmental samples from milk powders and infant formulas industries (except surface swabs)

Steps – negative samples	Alternative method	Reference method
Suspension preparation	D1	D1
Amplification	D2	/
PCR reading	D2	/
mLST inoculation	/	D2
Selective agar media isolation	/	D3
Petri dishes reading	/	D4

Steps – positive samples	Alternative method	Reference method
Suspension preparation	D1	D1
Amplification	D2	/
PCR reading	D2	/
mLST inoculation	D2	D2
Selective agar media isolation	D3	D3
Petri dishes reading	D4	D4
Purification on TSA	/	D4
Biochemical confirmation	/	D5
Reading	/	D6

Category: environmental samples from milk powders and infant formulas industries (**only surface swabs**)

Steps	Alternative method	Reference method
Suspension preparation	D1	D1
Amplification	D1	/
PCR reading	D1	/
mLST inoculation	/	D2
Selective agar media isolation	/	D3
Petri dishes reading	/	D4

Steps	Alternative method	Reference method
Suspension preparation	D1	D1
Amplification	D1	/
PCR reading	D1	/
mLST inoculation	D1	D2
Selective agar media isolation	D2	D3
Petri dishes reading	D3	D4
Purification on TSA	/	D4
Biochemical confirmation	/	D5
Reading	/	D6

10- Operator qualification
Identical as necessary for the reference method

11- Steps common with the reference method
None.

12- Traceability of analysis results
Traceability realized by the Sentinel software

13- Maintenance by laboratory
None.

3. Interlaboratory study

The main object of the collaborative study is to determine the variability of the results obtained by different laboratories analysing identical samples and to compare these results within the framework of the comparative study of the methods.

3.1. Collaborative study implementation

3.1.1. Participating laboratories

The collaborative study was realized by the expert laboratory and ten participating laboratories.

3.1.2. Absence of *Cronabacter* spp in the matrix

Before spiking, the absence of *Cronobacter* spp was verified in the batch of liquid infant formula used according to the reference method.

3.1.3. Strain stability in the matrix

The strain stability in the matrix was evaluated for 4 days at (5±3)°C. The strain used was *Cronobacter* spp (ISHA code : ENTB.3.44) isolated from the environment of a dairy industry.
A detection was performed after inoculation of 10 cells in 25 mL of matrix. An enumeration of *Cronobacter* spp was also made after an inoculation of 100 cells in 10 mL. The samples were analysed at D0, D+1, D+2 and D+3 by the reference method and by the alternative method. The results are summarized in table 9.

Day	Enumeration CFU/g	Alternative method	Reference method
D0	140	Presence in 50 mL	Presence in 50 mL
D+1	150	Presence in 50 mL	Presence in 50 mL
D+2	150	Presence in 50 mL	Presence in 50 mL
D+3	180	Presence in 50 mL	Presence in 50 mL

Tableau 9 : results of the stability study of the strain *Cronobacter* spp in infant formula

The results show that the *Cronobacter* strain used is stable for 3 days at (5±3)°C in the infant formula matrix.

3.1.4. Samples preparation and spiking

The matrix was inoculated with the target strain suspension to obtain 3 contamination levels:

-L0: 0 cell in 50 mL
-L1: 5 cells in 50 mL
-L2: 30 cells in 50 mL

The matrix was distributed at 50 mL in sterile vials. Every vial was individually spiked and homogenized. Eight samples per level, per laboratory and per method were prepared. Each laboratory received 48 samples to analyse, 1 sample to quantify the endogenous microflora and 1 water sample containing a temperature probe.
The results of the enumerations of the TVC, the target levels and the real levels of contamination are presented in table 10.

Matrix	Total viable count (CFU/mL)	Target level (cells / 50 mL)	Real level (cells / 50 mL)	Confidence interval
Infant formula	<10	0	0	0
		5	6	[2 ; 11]
		30	37	[26 ; 49]

Table 10: target level, real level and TVC of the matrix

3.1.5. Samples labeling

The labelling of the vials was realized as follows: a code to identify the laboratory: from A to J (cf. table 11) and a code to identify each sample, only known by the expert laboratory. The samples and

the temperature control vials (water sample with a temperature probe) were stored at 5°C before shipping.

Contamination level	Sample code
L0	2/6/7/10/13/16/21/24
L1	4/9/12/14/15/17/18/20
L2	1/3/5/8/11/19/22/23

Table 11: sample code by contamination level

3.1.6. Samples shipping

The samples were shipped in a coolbox the 4th of February 2013.

3.1.7. Samples reception and analysis

The coolboxes were received the 5th of February 2013 by all the participating laboratories. The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory. The samples were analysed the same day. The expert laboratory concurrently analysed a set of samples under the same conditions with both methods.

3.2. Results

3.2.1. Temperature and state of the samples

The temperature readings upon reception and the state of the samples are shown in table 12.

Laboratory	Temperature (°C)	State of the samples
A	8,7	Correct
B	5.4	Correct
C	4,9	Correct
D	5,7	Correct
E	5,8	Correct
F	3,2	Correct
G	6,0	Correct
H	6,2	Correct
I	3,7	Correct
J	6,5	Correct

Table 12: temperature and state of the samples upon reception

The temperature measurements are inferior to 8.4°C for all the laboratories, except for laboratory A for which it was at 8,7°C upon reception. However the temperature probe indicated a correct mean temperature between the shipping and the reception of the coolbox for this laboratory. The analysis of thermal profiles is shown in table 13.

Laboratory		A	B	C	D	E	F	G	H	I	J
Tempera-ture (°C)	Mean	6,5	4,1	2,0	5,1	5,6	2,6	3,2	2,7	4,1	4,2
	SD	1,5	2,4	3,8	1,0	2,0	1,0	1,8	0,7	1,1	1,8

Table 13: data of the temperature probes for the transportation time of samples

The thermal profiles analysis indicates for all laboratories mean temperatures comprises between 2.0 and 6.5°C.

3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 30°C vary between <10 and 17 000 CFU/mL.

3.2.3. Expert laboratory results

The results obtained by the expert laboratory are summarized in table 14.

Contamination level	Alternative method	Reference method (*)
L0	0/8	0/8
L1	8/8	8/8
L2	8/8	8/8

Table 14: positive results obtained by expert laboratory by both methods

The results are consistent with those expected.

3.2.4. Participating laboratories results

The results are summarized in tables 15 and 16. Raw results in appendix 7.

- Alternative method results

Laboratory	Contamination level		
	L0	L1	L2
A	0/8	8/8	8/8
B	0/8	7/8	8/8
C	0/8	8/8	8/8
D	0/8	8/8	8/8
E	0/8	7/8	8/8
F	0/8	8/8	8/8
G	0/8	8/8	8/8
H	0/8	8/8	8/8
I	0/8	8/8	8/8
J	0/8	8/8	8/8
TOTAL	**0/80**	**78/80**	**80/80**

Table 15: alternative method positive results for all laboratories

- Reference method results

Laboratory	Contamination level		
	L0	L1	L2
A	0/8	8/8	8/8
B	0/8	8/8	8/8
C	0/8	8/8	8/8
D	0/8	8/8	8/8
E	0/8	7/8	8/8
F	0/8	8/8	8/8
G	0/8	8/8	8/8
H	1/8	8/8	8/8
I	0/8	6/8	8/8
J	1/8	8/8	8/8
TOTAL	**2/80**	**77/80**	**80/80**

Table 16: reference method positive results for all laboratories

- Results analysis

Contamination level L0

Labs H and J showed a negative result by the alternative method and positive by the reference method for two non spiked samples (negative deviation for samples H10 and J24). A second confirmation of the reference method confirmed the presence of Cronobacter spp in the enrichment broth. These positive results could be due to a cross-contamination.

Contamination level L1

The laboratory B showed a negative result by the alternative method and a positive result by the reference method for sample B20. The confirmation and another validated PCR method were applied. These tests showed the absence of Cronobacter spp in the broth.
As the test sample is different for the two methods and the inoculation level is low, it's possible that no cell of Cronobacter spp was present in the test sample analyzed.

Lab E showed a negative result with both methods for sample E15. Enrichment broths were tested twice by the confirmation methods and always gave a negative result. As the inoculation level is low, it's possible that no cell of Cronobacter spp was present in the samples analyzed.

Lab I had two samples giving a positive result by the alternative method and a negative result by the reference method (I12 and I18).Complementary analyses were asked by the expert lab but were not performed by the laboratory.
However, as the inoculation level is low, it's possible that no cell of *Cronobacter* spp was present in the sample analyzed.

3.4.1. Specificity (SP) and sensitivity (SE) calculations

The specificity and sensitivity calculations of both methods are presented in table 17, with the low critical value (LCL). Formulas used are:

For level L0, SP = [1-(FP/N-)] x 100%, N-: total number of L0 tests
FP: number of false positive

For levels L1 and L2, SE = (TP/N+) x 100%, N+: total numbers of L1 or L2 tests
TP: number of true positive

Specificity / sensitivity	Alternative method	LCL	Reference method	LCL
SP (level L0)	100,0%	98,0%	97,5%	96,0%
SE (level L1)	97,5%	96,0%	96,3%	93,0%
SE (level L2)	100,0%	98,0%	100%	98,0%
SE (level L1+L2)	98,8%	98,0%	98,2%	96,0%

Table 17: specificity (SP), sensitivity (SE) and LCL of alternative and reference method

3.4.2. Relative accuracy calculations

Pairs of results of the different levels of contamination are presented in table 18.

Level	Alternative method	Reference method		
		RM+	**RM-**	**Total**
L0	**MA+**	PA=0	PD=0	0
	MA-	ND=2	NA=78	80
	Total	2	78	80
L1	**MA+**	PA=76	PD=2	78
	MA-	ND=1	NA=1	2
	Total	77	3	80
L2	**MA+**	PA=80	PD=0	80
	MA-	ND=0	NA=0	0
	Total	80	0	80
L0+L1+L2	**MA+**	PA=156	PD=2	158
	MA-	ND=3	NA=79	82
	Total	159	81	240

Table 18: tests results for both methods (PA: positive agreement, NA: negative agreement, ND: negative deviation, PD: positive deviation)

Relative accuracy values of the different contamination levels are presented in table 19 with their LCL. Formula used is the following:
AC = (PA+NA)/N x 100%, PA: number of positive agreements
NA: number of negative agreements

Level	Relative accuracy (AC)	LCL (Low Critical Value)
L0	97,5%	96,0%
L1	96,3%	93,0%
L2	100,0%	98,0%
L1+L2	98,1%	96,0%
Total	97,9%	96,0%

Table 19: relative accuracy values (AC) and LCL of alternative method

3.4.3. Discordant results analysis

Discordant results are analysed according to the annex F of ISO 16140 standard. The total number of discordant results is given by the following formula: Y = PD + ND.
In the present case, Y = 2 + 3 = 5, so with Y < 6, the two methods are considered as equivalent.

3.4. Interpretation

3.4.1. Accordance

The accordance is the percentage chance of finding the same result (i.e. both negative or both positive) from two identical test portions analyzed in the same laboratory, under repeatability conditions (i.e. one operator using the same apparatus and same reagents within the shortest feasible time interval).

To derive the accordance from the results of an interlaboratory study, the probability that two samples give the same result is calculated for each participating laboratory in turn, and this probability is then averaged over all laboratories. Values of accordance are shown in table 20. Calculations of accordance by level and method are presented in appendix 8.

Level	Alternative method	Reference method
L0	100,0%	95,6%
L1	95,6%	94,1%
L2	100,0%	100,0%

Table 20 : accordance by level and method

3.4.2. Concordance

The concordance is the percentage chance of finding the same result for two identical samples analysed in two different laboratories.

To calculate the concordance from the results of an interlaboratory study, take in turn each replicate in each participating laboratory, pair it with identical results of all the other laboratories. The concordance is the percentage of all pairings giving the same results on all the possible pairings of data. Values of concordance are shown in table 21. Calculations of concordance by level and method are presented in appendix 9.

Level	Alternative method	Reference method
L0	100,0%	95,1%
L1	95,1%	92,6%
L2	100,0%	100,0%

Table 21 : concordance by level and method

3.4.3. Concordance odds ratio

If the concordance is smaller than the accordance, it indicates that two identical samples are more likely to give the same result if they are analysed by the same laboratory than if they are analysed by different ones, suggesting that there can be variability in performance between laboratories. Unfortunately, the magnitude of the concordance and accordance is strongly dependent on the level of accuracy, making it difficult to assess easily the degree of between-laboratory variation.

It is therefore helpful to calculate the concordance odds ratio (COR) defined as follows:
COR = accordance x (100-concordance)/concordance x (100 – accordance).

Values of COR for both methods are shown in table 22.

A value for the odds ratio of 1.00 would be expected if accordance and concordance were equal, and the larger the odds ratio is, the more inter-laboratory variation is predominant. Nevertheless, values above 1.00 can occur by chance variation, and so a statistical significance test should be used to confirm whether the evidence for extra variation between laboratories is convincing. The "exact test" is the best recommended test for this). The philosophy behind such tests is that the probabilities of occurrence are calculated for all sets of replicate results that could have produced the overall numbers of positives and negatives.

Niveau de contamination	**Méthode alternative**			**Méthode de référence**		
	Degré d'accord	**Concordance**	**COR**	**Degré d'accord**	**Concordance**	**COR**
L0	100,0	100,0	1,0	95,6	95,1	1,1
L1	95,6	95,1	1,1	94,1	92,6	1,3
L2	100,0	100,0	1,0	100,0	100,0	1,0

Table 22 : COR values for each method by contamination level

3.4.4. AC, SP, SE comparison

Table 23 summarizes the values obtained for AC, SP and SE parameters for the preliminary study and the interlaboratory study.

Parameter	**Preliminary study**	**Interlaboratory study**
AC	97,4%	97,5%
SP	98,2%	100%
SE	96,7%	98,8%

Table 23 : AC, SP and SE comparison between preliminary and interlaboratory study

The values obtained during the collaborative study are better than those obtained during the preliminary study, probably because of the greater variety of samples and strains tested during the preliminary study.

4. Conclusion

- Comparative study

The performances of the ADIAFOOD *Cronobacter* spp test are comparable to those of the standard ISO/TS 22964.
This study concerned 235 samples of three categories of products:

- milk powders / infant formulas / dehydrated complements
- matrices used in the composition of milk powders and infant formulas
- environmental samples from milk powders and infant formulas industries

Values obtained for the 3 criteria are the following:

-relative accuracy: 97.4%
-relative sensitivity: 98.2%
-relative specificity: 96.7 %

Some discordant results were observed (4 PD and 2 ND). These discordances may be linked to the sensitivity of the reference method, to the sampling which is different between the two methods and to the volumes used in the alternative method confirmation protocol.

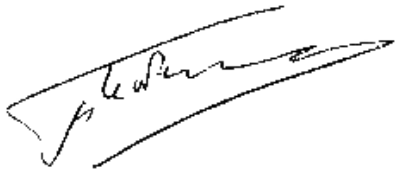
The relative level of detection of the alternative method and the reference method was evaluated for all categories. The results are comparable between the two methods. It varies between 0.5 and 1.7 CFU in 50 g for the reference method and between 0.5 and 1.5 CFU in 50 g for the alternative method for all categories.

The specificity of the method is satisfactory.

The study of the practicability of the alternative method shows a simple and easy-to-use method and a significant time savings compared to the reference method.

- Interlaboratory study

Concerning the interlaboratory study, the results obtained for the selected laboratories showed that the values of relative accuracy, relative sensitivity and relative specificity are better to those obtained during the preliminary study due to the higher diversity of samples tested during the comparative study. The variability of the alternative method, demonstrated by the calculations of accordance, concordance and concordance odds ratio, is similar to that of the reference method.



Massy, the 10th of December 2013
François Le Nestour
Responsable de l'Unité Innovation Biologie

APPENDIX 1

ALTERNATIVE METHOD PROTOCOL

STEP 1 : ENRICHMENT

Dilution at 1/10th in a full filter bag of 50 g or 50 mL of sample in buffered peptone water (with a pre-heating at 45°C if an homogenous suspension can be obtained even after mixing)

Category milk powders / infant formulas / dehydrated complements:
Add the selective supplement, mix by manual agitation
Incubation at 37±1 °C for 18±2 h

Category matrices used in the composition of milk powders and infant formulas
Category environmental samples from milk powders and infant formulas industries (**except surface swabs**)
Mix by manual agitation
Incubation at 37±1 °C for 18±2 h

Category environmental samples from milk powders and infant formulas industries (**only surface swabs**)
Mix by manual agitation
Incubation at 37±1 °C for 8 to 24 h

STEP 2 : DNA EXTRACTION

Sample 1 mL of enriched broth in a 1,5 mL tube
Place 10 µL of this suspension + 90 µL of EX2 buffer in an extraction tube
Extraction in thermocycler

STEP 3 : DNA AMPLIFICATION

Mix 15 µL of DT2 solution with 10 µL of extract
Detection in thermocycler

STEP 4 : RESULTS

Display of the results on the screen
Optional protocol to lift the inhibitions by dilution of the extract at 1/10th

STEP 5 : CONFIRMATION OF PRESUMED POSITIVE SAMPLES

Add 500 µL of primary enrichment in 10 mL of mLST broth
Incubation at 44±0,5 °C for 24±2 h
Isolation on ESIA agar and incubation at 44±1 °C for 24±2 h
And isolation on ChromID Sakazakii agar and incubation at 37±1 °C for 24±3 h
Reading of the Petri dishes

APPENDIX 2

REFERENCE METHOD PROTOCOL

Reference method ISO/TS 22964

ENRICHMENT

X g (mL) sample + 9 X mL buffered peptone water
Incubation at (37±1)°C for 16 to 20 hours

SELECTIVE ENRICHMENT

Transfer 0,1 mL enriched BPW in 10 mL mLST broth
Incubation in water bath at (44±0,5)°C for 22 to 26 hours

SELECTIVE ISOLATION

Make an isolation on ESIA agar media
Incubation at (44±1)°C for 22 to 26 heures

PURIFICATION

1 to 5 typical colonies
Isolation on TSA
Incubation at (25±1)°C for 44 to 48 hours

CONFIRMATION

Biochemical confirmation

EXPRESSION OF THE RESULTS

Appendix 3- Bacterial stressed cells

Produits laitiers

N° échantillon	Code souche	Souche	Origine	Stress appliqué	Intensité du stress
AC13	ENTB 3.16	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C/24h à 4°C +5min à 56°C/24h à 4°C + 10 min à 56°C	0,5
AC14	ENTB 3.16	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C/24h à 4°C +5min à 56°C/24h à 4°C + 10 min à 56°C	0,5
AC15	ENTB 3.16	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C/24h à 4°C +5min à 56°C/24h à 4°C + 10 min à 56°C	0,5
AC127	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	2,5
AC128	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	2,5
AC144	ENTB 3.32	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	0,7
AC209	ENTB 3.25	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,5
AC210	ENTB 3.25	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,5
AC211	ENTB 3.25	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,5
AC213	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	0,6
AC214	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	0,6
AC216	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	1,1
AC1	ENTB.3.1	*Cronobacter sakazakii subsp sakazakii*	Poudre de lait	25 min à 65°C	0,5
AC2	ENTB.3.1	*Cronobacter sakazakii subsp sakazakii*	Poudre de lait	25 min à 65°C	0,5
AC3	ENTB.3.1	*Cronobacter sakazakii subsp sakazakii*	Poudre de lait	25 min à 65°C	0,5
AC7	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	10 min à 51°C	0,5
AC8	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	10 min à 51°C	0,5
AC9	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	10 min à 51°C	0,5
AC31	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	10 min à 51°C	0,5
AC34	ENTB 3.33	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	45 min à 56°C	0,6
AC35	ENTB 3.33	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	45 min à 56°C	0,6
AC36	ENTB 3.33	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	45 min à 56°C	0,6
AC125	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	0,5
AC 129	ENTB 3.43	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	2,5
AC130	ENTB 3.43	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	2,5
AC131	ENTB 3.43	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	2,5
AC132	ENTB 3.43	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C + eau froide 10 min	2,5
AC133	ENTB 3.22	*Cronobacter sakazakii*	CIP 103183	20 min à 55°C + eau froide 10 min	0,5
AC134	ENTB 3.22	*Cronobacter sakazakii*	CIP 103183	20 min à 55°C + eau froide 10 min	0,5
AC135	ENTB 3.22	*Cronobacter sakazakii*	CIP 103183	20 min à 55°C + eau froide 10 min	0,5
AC136	ENTB 3.22	*Cronobacter sakazakii*	CIP 103183	20 min à 55°C + eau froide 10 min	0,5
AC217	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	1,1
AC218	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	1,1
AC263	ENTB 3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	1,1
AC265	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 65°C	1,6
AC266	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	15 min à 65°C	1,7
AC267	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	15 min à 65°C	1,7
AC268	ENTB 3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	15 min à 65°C	1,2
AC269	ENTB 3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	15 min à 65°C	1,2
AC270	ENTB 3.42	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	1,7
AC271	ENTB 3.26	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	1,7
AC264	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 65°C	1,6

Produits divers

N° échantillon	Code souche	Souche	Origine	Stress appliqué	Intensité du stress
AC49 SN	ENTB 3.2	*Cronobacter sakazakii*	CIP 57.33	35 min à 55°C	2,6
AC50 SN	ENTB 3.2	*Cronobacter sakazakii*	CIP 57.33	35 min à 55°C	2,6
AC59SN	ENTB 3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,7
AC60 SN	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	1,5
AC151	ENTB 3.10	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	1,0
AC158	ENTB 3.2	*Cronobacter sakazakii*	CIP 57.33	35 min à 55°C	2,6
AC159	ENTB 3.2	*Cronobacter sakazakii*	CIP 57.33	35 min à 55°C	2,6
AC160	ENTB 3.2	*Cronobacter sakazakii*	CIP 57.33	35 min à 55°C	2,6
AC168	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	1,5
AC170	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	1,5
AC171	ENTB 3.35	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 60°C	1,1
AC173	ENTB 3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 58°C	0,6
AC174	ENTB 3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 58°C	0,6
AC175	ENTB 3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 58°C	0,6
AC51 SN	ENTB 3.15	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	35 min à 55°C	0,6
AC54 SN	ENTB 3.15	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	35 min à 55°C	0,6
AC65SN	ENTB 3.35	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 60°C	1,1
AC147	ENTB 3.15	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	35 min à 55°C	0,6
AC152	ENTB 3.10	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	1,0
AC153	ENTB 3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,7
AC166	ENTB 3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,7
AC167	ENTB 3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,7
AC169	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	1,5
AC172	ENTB 3.35	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 60°C	1,1
AC176	ENTB 3.10	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	1,0
AC177	ENTB 3.10	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	1,0
AC178	ENTB 3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,7
AC179	ENTB 3.21	*Cronobacter muytjensii*	CIP 103581	5 min à 60°C	1,5
AC181	ENTB 3.35	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 60°C	1,1
AC182	ENTB 3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 58°C	0,6
AC183	ENTB 3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 58°C	0,6
AC184	ENTB 3.39	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C	0,7
AC185	ENTB 3.39	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C	0,7
AC186	ENTB 3.39	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C	0,7
AC187	ENTB 3.39	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 55°C	0,7
AC190	ENTB 3.41	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,5
AC191	ENTB 3.41	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	5 min à 60°C	0,5
AC192	ENTB 3.11	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,5
AC193	ENTB 3.11	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,5
AC194	ENTB 3.11	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,5
AC195	ENTB 3.11	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,5
AC196	ENTB 3.19	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,9
AC197	ENTB 3.19	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,9
AC198	ENTB 3.19	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,9
AC199	ENTB 3.19	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C	0,9
AC57 SN	ENTB 3.16	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10 min à 60°C/24h à 4°C +5min à 56°C/24h à 4°C + 10 min à 56°C	0,5

Echantillons d'envirronnement

N° échantillon	Code souche	Souche	Origine	Stress appliqué	Intensité du stress
AC272	ENTB3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC273	ENTB3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC275	ENTB3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC276	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C	1,1
AC277	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C	1,1
AC278	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C	1,1
AC279	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C	1,1
AC280	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC281	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC282	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC283	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C	0,5
AC248	ENTB 3.51	*Cronobacter turicensis*	ADRIA developpement	20 min à 65°C	0,7
AC231	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 65°C	1,6
AC232	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15 min à 65°C	1,6
AC233	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	15 min à 65°C	1,7
AC234	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	15 min à 65°C	1,7
AC235	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	15 min à 65°C	1,7
AC236	ENTB 3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	15 min à 65°C	1,7
AC237	ENTB 3.1	*Cronobacter sakazakii subsp sakazakii*	poudre de lait	15 min à 65°C	1,6
AC238	ENTB 3.1	*Cronobacter sakazakii subsp sakazakii*	poudre de lait	15 min à 65°C	1,6
AC239	ENTB 3.1	*Cronobacter sakazakii subsp sakazakii*	poudre de lait	15 min à 65°C	1,6
AC240	ENTB 3.1	*Cronobacter sakazakii subsp sakazakii*	poudre de lait	15 min à 65°C	1,6
AC242	ENTB 3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	15 min à 65°C	1,2
AC244	ENTB 3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	15 min à 65°C	1,2
AC246	ENTB 3.51	*Cronobacter turicensis*	ADRIA developpement	20 min à 65°C	0,7
AC249	ENTB 3.42	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	1,7
AC250	ENTB 3.42	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	1,7
AC251	ENTB 3.42	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	1,7
AC254	ENTB 3.26	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	3,7
AC256	ENTB 3.26	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	3,7
AC258	ENTB 3.26	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	20 min à 65°C	3,7
AC96 C	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C + 15 jrs à 4°C	0,5
AC97 C	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C + 15 jrs à 4°C	0,5
AC98 C	ENTB 3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pastyeur de Lille	25 min à 65°C + 15 jrs à 4°C	0,5
AC100 C	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	1,1
AC101 C	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	1,1
AC102 C	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	1,1
AC103 C	ENTB 3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	1,1
AC104 C	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	0,5
AC105C	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	0,5
AC106C	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	0,5
AC107C	ENTB 3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	25 min à 65°C + 15 jrs à 4°C	0,5
AC335	ENTB.3.43	*Cronobacter spp*	Environnement industrie laitière	20 min à 56°C	0,5
AC336	ENTB.3.43	*Cronobacter spp*	Environnement industrie laitière	20 min à 56°C	0,5
AC337	ENTB.3.43	*Cronobacter spp*	Environnement industrie laitière	20 min à 56°C	0,5
AC338	ENTB.3.43	*Cronobacter spp*	Environnement industrie laitière	20 min à 56°C	0,5
AC340	ENTB.3.43	*Cronobacter spp*	Environnement industrie laitière	20 min à 56°C	0,5
AC341	ENTB.3.19	*Cronobacter spp*	Environnement industriel	20 min à 56°C	0,8
AC342	ENTB.3.19	*Cronobacter spp*	Environnement industriel	20 min à 56°C	0,8
AC343	ENTB.3.19	*Cronobacter spp*	Environnement industriel	20 min à 56°C	0,8
AC344	ENTB.3.19	*Cronobacter spp*	Environnement industriel	20 min à 56°C	0,8
AC345	ENTB.3.19	*Cronobacter spp*	Environnement industriel	20 min à 56°C	0,8
AC355	ENTB.3.14	*Cronobacter spp*	Environnement industriel	10 min à 51°C	0,5
AC356	ENTB.3.14	*Cronobacter spp*	Environnement industriel	10 min à 51°C	0,5
AC357	ENTB.3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	15 min à 51°C	1,0
AC358	ENTB.3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	15 min à 51°C	1,0
AC359	ENTB.3.20	*Cronobacter spp*	Environnement industriel	20 min à 51°C	0,5
AC360	ENTB.3.20	*Cronobacter spp*	Environnement industriel	20 min à 51°C	0,5
AC361	ENTB.3.13	*Cronobacter spp*	Environnement industriel	20 min à 51°C	0,5
AC362	ENTB.3.13	*Cronobacter spp*	Environnement industriel	20 min à 51°C	0,5
AC363	ENTB.3.6	*Cronobacter spp*	Environnement	20 min à 51°C	0,6
AC364	ENTB.3.6	*Cronobacter spp*	Environnement	20 min à 51°C	0,6

APPENDIX 4

RELATIVE ACCURACY

RAW RESULTS

+: positive result or presence of typical colonies
-: negative result or absence of typical colonies
/: test not realized
ND: negative deviation
PD: positive deviation
PP: presumed positive before confirmation
=: positive agreement (PA) or negative (NA) between AM et RM
0 : absence of growth
** : sample inhibited in first analysis, then diluted at one tenth
NC : naturally contaminated

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP- EXACTITUDE RELATIVE

Produits laitiers - Echantillons négatifs

n° echant	Produit	Taux d'inocu-lation (UFC / 50g)	MA: ADIAFOOD Cronobacter						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA
			PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final	
					Chrom ID sakazakii	ESIA	Galerie					
AC13	Poudre de lait VW13	1	-					-	-		-	=
AC14	Poudre de lait VW14	1	-					-	-		-	=
AC15	Poudre de lait VW15	1	-					-	-		-	=
AC16	Poudre de lait VW16		-					-	-		-	=
AC17	Poudre de lait VW17		-					-	-		-	=
AC18	Poudre de lait VW18		-					-	-		-	=
AC19	Poudre de lait VW19		-					-	-		-	=
AC20	Poudre de lait VW20		-					-	-		-	=
AC21	Lait en poudre		-					-	-		-	=
AC23	Alimentation infantile		-					-	-		-	=
AC127	Echantillon 1 evolia relais (*L.reuteri*)	9	-					-	0		-	=
AC128	Echantillon 2 (*S.thermophilus, B.lactis*)	9	-					-	0		-	=
AC144	Echantillon 3		-					-	0		-	=
AC200	Echantillon 4 criossance 3		-					-	0		-	=
AC201	Echantillon 5		-					-	0		-	=
AC202	Echantillon 6 lait 2eme age		-					-	0		-	=
AC203	Echantillon 7		-					-	0		-	=
AC204	Echantillon 24 (Bifidobacterie)		-					-	0		-	=
AC205	Echantillon 25 (*L.reuteri*)		-					-	0		-	=
AC206	Echantillon 8 confort premium (Bifidobacterie)		-					-	0		-	=
AC207	Echantillon 26 (Bifidobacterie)		-					-	0		-	=
AC208	Echantillon 9 lait lait écrémé en poudre		-					-	0		-	=
AC209	Echantillon 6 céréales	2	-					-	0		-	=
AC210	Echantillon 10 (*S.thermophilus,B.lactis*)	2	-					-	0		-	=
AC211	Echantillon 11	2	-					-	0		-	=
AC213	Echantillon 12 céréales et vanille	2	-					-	-		-	=
AC214	Echantillon 13 (L.rhamnosus, B.infantis)	2	-					-	+	Ent.amnigenus	-	=
AC216	Echantillon 27 (*L.reuteris*)	3	+	33,9	0	0		-	0		-	= (PPNA)
AC259	Echantillon 14		-					-	0		-	=
A260	Echantillon 15		-					-	0		-	=
AC261	Echantillon 28		-					-	0		-	=
AC262	Echantillon 29 naissance 1 (*S.Thermophilus, B.lactis*)		-					-	0		-	=

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP- EXACTITUDE RELATIVE

Produits laitiers - Echantillons positifs

n° echant	Produit	Taux d'inocu-lation (UFC / 50g)	MA: ADIAFOOD Cronobacter						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA
			PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final	
					Chrom ID sakazakii	ESIA	Galerie					
AC1	Echantillon 17	17	+	20,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC2	Echantillon 18 lait de croissance céréales pour bébé	17	+	22,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC3	Poudre de lait VW3	17	+	21,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC7	Echantillon 19 lait 2éme age	20	+	30,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC8	Echantillon 30 lait de croissance	20	+	28,5	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC9	Poudre de lait VW9	20	+	31,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC31	Alimentation infantile	22	+	25,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC34	Lait en poudre VW17	20	+	33,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC35	Echantillon 31 lait de croissance	20	+	27,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC36	Echantillon 20 lait de croissance	20	+	26,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC125	Echantillon 32 chocovanille (*Bifidobacterium lactis*)	9	+	17,2	+	+	Ent.sakazakii	+	0		-	≠ (PD)
AC 129	Echantillon 33 (Lactobacillus *Reuteri*)	22	+	23,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC130	Echantillon 12 lait écréme poudre	22	+	31,2	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC131	Echantillon 21 (*Bifidobacterium*)	22	+	22,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC132	Echantillon 26 (Bifidobacteries)	22	+	18,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC133	Echantillon 10 (Bifidobacterium lactis)	21	+	17,9	+	+	Ent.sakazakii	+	0		-	≠ (PD)
AC134	Echantillon 34 (Bifidobacteries)	21	+	17,2	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC135	Echantillon 8 (Bifidobacterie)	21	+	17,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC136	Echantillon 21 (*Bifidobacterium*)	21	+	18,5	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC217	Echantillon 35 (Bifidobacteries)	3	+	16,9	+	+	Ent.sakazakii	+	0			≠ (PD)
AC218	Echantillon 37 lait écréme poudre	3	+	17,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC263	Echantillon 36 céréales vanille	5	+	18,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC265	Echantillon 22 compléments céréales infantiles lactées	5	+	20,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC266	Echantillon 38 lait croissance	7	+	24,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC267	Echantillon 27 lait infantile 1er age	7	+	26,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC268	Echantillon 39 lait 2eme age	8	+	18,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC269	Echantillon 23	8	+	21,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC270	Echantillon 40	6	+	17,2	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC271	Echantillon 41	6	+	29,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC264	Echantillon 29 naissance 1 (S.Thermophilus, B.lactis)	5	-		+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	≠ (ND)

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP- EXACTITUDE RELATIVE

Produits divers - Echantillons négatifs

n° echant	Produit	Taux d'inocu-lation (UFC / 50g)	MA: ADIAFOOD Cronobacter						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA
			PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final	
					Chrom ID sakazakii	ESIA	Galerie					
AC88	Protéine entière protarmor 80 AK1		-					-	0		-	=
AC89	Protéine hydrolysée peptigen IF3012		-					-	0		-	=
AC91	Carbohydrate amidon de manioc AX		-					-	0		-	=
AC93	Protéine hydrolysée lacprodan Di3071 AY2		-					-	0		-	=
AC94	Protéine hydrolysée lacprodan Di3071 AY1		-					-	0		-	=
AC95	Protéine entière protarmor 80 AK2		-					-	0		-	=
AC49 SN	Carbohydrate sucre cristtal M	2	-					-	0		-	=
AC50 SN	Carbohydrate lactose edible H	2	-					-	0		-	=
AC59SN	Protéine entière WPC60 I2	1	-					-	-		-	=
AC60 SN	Protéine entière lacprodan alpha10	2	-					-	-		-	=
AC151	Carbohydrate lactose edible AAE	2	-					-	0		-	=
AC158	Carbohydrate lactose edible L	6	-					-	0		-	=
AC159	Carbohydrate lactose edible H	6	-					-	0		-	=
AC160	Protéine entière LPN isolat de protéine D3	6	-					-	0		-	=
AC168	Ajout à sec fructose G	9	-					-	0		-	=
AC170	Carbohydrate lactose edible S	9	-					-	0		-	=
AC171	Protéine entière lacprodan di8050	3	-					-	0		-	=
AC173	Protéine entière lactoserum déminé 90% A	2	-					-	0		-	=
AC174	Protéine entière lacprodan alpha10 AE2	2	-					-	0		-	=
AC175	Protéine entière isolat de protéine D1	2	-					-	0		-	=
AC219	Ajout à sec Malto de 19 AR		-					-	0		-	=
AC220	Protéine entière lactoserum déminé 90% AP		-					-	0		-	=
AC221	Carbohydrate amidon de manioc AX		-					-	0		-	=
AC222	Carbohydrate amidon de tapioca AW		-					-	0		-	=
AC223	Ajout à sec malto de 19 AAAH1		-					-	0		-	=
AC224	Carbohydrate lactose pharmaceutique AN		-					-	0		-	=
AC225	Protéine entière protilight AAA2		-					-	0		-	=
AC226	Carbohydrate fructose standard AO		-					-	0		-	=
AC227	Lactose saka free U		-					-	0		-	=
AC228	Carbohydrate lactose edible S		-					-	0		-	=
AC229	Ajout à sec lactose		-					-	0		-	=
AC230	Protéine entiére		-					-	0		-	=

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP- EXACTITUDE RELATIVE

Produits divers - Echantillons positifs

n° echant	Produit	Taux d'inocu-lation (UFC / 50g)	MA: ADIAFOOD Cronobacter						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA
			PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final	
					Chrom ID sakazakii	ESIA	Galerie					
AC51 SN	Ajout à sec lactose K	3	+	25,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC54 SN	Carbohydrate lactose edible L	3	+	27,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC65SN	Protéine entière LPN isolat de protéine D2	1	+	17,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC147	Carbohydrate lactose pharmaceutique AN	3	+	19,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC152	Ajout à sec lactose U	2	+	18,3	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC153	Protéine entière protilight AAA1	1	+	18,3	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC166	Protéine entière WPC60 I1	4	+	24,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC167	Carbohydrate sucre cristal M	4	+	21,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC169	Ajout à sec lactose K	9	+	21,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC172	Carbohydrate lactose edible O	3	+	25,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC176	Carbohydrate dextrose I monohydrate J	4	+	18,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC177	Protéine hydrolysée lacprodan Di3071 E1	4	+	18,5	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC178	Protéine entière lacprodan Di8090 F1	3	+	24,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC179	Lait de Soja 1	2	+	20,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC181	Protéine entière caséinate de potassium N2	2	+	19,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC182	Protéine hydrolysée lacprodan Di2021 C1	1	-		+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	≠ (ND)
AC183	Protéine WPC60 AL1	1	+	33,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC184	Carbohydrate lactose edible S	6	+	18,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC185	Protéine hydrolysée lacprodan Di2021 C2	6	+	22,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC186	Carbohydrate amidon de manioc AX	6	+	27,2	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC187	Protéine entière WPC60 I2	6	+	18,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC190	Protéine entière lactoserum déminé 90% BB A	19	+	20,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC191	Protéine hydrolysée peptigen IF3012 AZ1	19	+	16,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC192	Carbohydrate sirop de glucose AA	10	+	18,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC193	Protéine entière caséine de potassium N1	10	+	18,3	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC194	Echantillon 42	10	+	19,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC195	Protéine hydrolysée peptigen IF3012 AZ2	10	+	17,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC196	Lait de soja 2	8	+	21,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC197	Protéine hydrolysée lacprodan Di3071 AY1	8	+	18,5	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC198	Carbohydrate lactose edible H	8	+	17,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC199	Protéine WPC60 AL2	8	+	24,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=
AC57 SN	Carbohydrate dextrose I monohydrate	2	+	30,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP- EXACTITUDE RELATIVE

Echantillons d'environnements (échantillons de surfaces) - Echantillons négatifs

n° echant	Produit	Taux d'inocu-lation (UFC / 50g)	MA: ADIAFOOD Cronobacter 8h						MA: ADIAFOOD Cronobacter 24h						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA 8h	Concordance MR /MA 24h
			PCR	Ct	Confirmation			Résultat Final	PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final		
					Chrom ID sakazakii	ESIA	Galerie				Chrom ID sakazakii	ESIA	Galerie						
AC96	Industrie laitiére 1		-					-	-					-	0		-	=	=
AC97	Industrie laitiére 2		-					-	-					-	0		-	=	=
AC98	Industrie laitiére 3		-					-	-					-	0		-	=	=
AC99	Industrie laitiére 4		-					-	-					-	0		-	=	=
AC100	Industrie laitiére 5		-					-	-					-	-		-	=	=
AC101	Industrie laitiére 6		-					-	-					-	0		-	=	=
AC102	Industrie laitiére 7		-					-	-					-	-		-	=	=
AC103	Industrie laitiére 8		-					-	-					-	0		-	=	=
AC104	Industrie laitiére 9		-					-	-					-	0		-	=	=
AC105	Industrie laitiére 10		-					-	-					-	-		-	=	=
AC106	Industrie laitiére 11		-					-	-					-	0		-	=	=
AC107	Industrie laitiére 12		-					-	-					-	0		-	=	=
AC108	Industrie laitiére 13		-					-	-					-	0		-	=	=
AC109	Industrie laitiére 14		-					-	-					-	0		-	=	=
AC110	Industrie laitiére 15		-					-	-					-	0		-	=	=
AC112	Industrie laitiére 17		-					-	-					-	0		-	=	=
AC113	Industrie laitiére 18		-					-	-					-	0		-	=	=
AC114	Industrie laitiére 19		-					-	-					-	0		-	=	=
AC115	Industrie laitiére 20		-					-	-					-	-		-	=	=
AC116	Industrie laitiére LM3 bac gastro CGA 2404		-					-	-					-	0		-	=	=
AC117	Industrie laitiére LM1		-					-	-					-	-		-	=	=
AC118	Industrie laitiére DE1		-					-	-					-	-		-	=	=
AC272	Industrie laitiére 19 2D (lingette)	8	-					-	-					-	-		-	=	=
AC273	Industrie laitiére 18 2H	8	-					-	-					-	0		-	=	=
AC275	Industrie laitiére 23 4E (lingette)	8	-					-	-					-	0		-	=	=
AC276	Industrie laitiére 27 bis	4	-					-	-					-	0		-	+	+
AC277	Industrie laitiére 45	4	-					-	-					-	0		-	=	=
AC278	Industrie laitiére 51	4	-					-	-					-	-		-	=	=
AC279	Industrie laitiére 65	4	-					-	-					-	0		-	=	=
AC280	Industrie laitiére 67	5	-					-	-					-	0		-	=	=
AC281	Industrie laitiére 281	5	-					-	-					-	0		-	=	=
AC282	Industrie laitiére 74	5	-					-	-					-	0		-	=	=
AC283	Industrie laitiére 78	5	+	35	0	0		-	-					-	0		-	= (PPNA)	= (PPNA)

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP- EXACTITUDE RELATIVE

Echantillons d'environnements (échantillons de surfaces) - Echantillons positifs

n° echant	Produit	Taux d'inocu-lation (UFC / 50g)	MA: ADIAFOOD Cronobacter 8h						MA: ADIAFOOD Cronobacter 24h						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA 8h	Concordance MR /MA 24h
			PCR	Ct	Confirmation			Résultat Final	PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final		
					Chrom ID sakazakii	ESIA	Galerie				Chrom ID sakazakii	ESIA	Galerie						
AC231	Industrie laitiére 25	3	+	34,0	+	+	Ent.sakazakii	+	+	25,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC232	Industrie laitiére 26	3	+	34,9	+	+	Ent.sakazakii	+	+	26,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC233	Industrie laitiére 27	3	+	34,2	+	+	Ent.sakazakii	+	+	29,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC234	Industrie laitiére 28	3	+	30,4	+	+	Ent.sakazakii	+	+	18,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC235	Industrie laitiére 29	3	+	34,1	+	+	Ent.sakazakii	+	+	26,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC236	Industrie laitiére 31	3	+	31,4	+	+	Ent.sakazakii	+	+	28,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC237	Industrie laitiére 32	5	+	30,5	+	+	Ent.sakazakii	+	+	28,1	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC238	Industrie laitiére 33	5	+	29,5	+	+	Ent.sakazakii	+	+	18,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC239	Industrie laitiére 34	5	+	30,6	+	+	Ent.sakazakii	+	+	28,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC240	Industrie laitiére 35	5	+	29,1	+	+	Ent.sakazakii	+	+	23,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	=	=	=
AC242	Industrie laitiére 36	5	+	34,2	+	+	Ent.sakazakii	+	+	28,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC244	Industrie laitiére 37	5	+	34,1	+	+	Ent.sakazakii	+	+	27,6	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC246	Industrie laitiére 38	7	+	34,5	+	+	Ent.sakazakii	+	+	22,4	+	+	Ent.sakazakii	+	0		-	≠ (PD)	≠ (PD)
AC249	Industrie laitiére 39	4	+	33,2	+	+	Ent.sakazakii	+	+	19,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC250	Industrie laitiére 40	4	+	33,6	+	+	Ent.sakazakii	+	+	16,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC251	Industrie laitiére 41	4	+	33,2	+	+	Ent.sakazakii	+	+	14,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC254	Industrie laitiére 42	5	+	34,0	+	+	Ent.sakazakii	+	+	16,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC256	Industrie laitiére 43	5	+	32,5	+	+	Ent.sakazakii	+	+	19,2	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC258	Industrie laitiére 44	5	+	32,3	+	+	Ent.sakazakii	+	+	20,5	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC96 C	Industrie laitiére 1 (lingette)	4	+	19,2	+	+	Ent.sakazakii	+	+	16,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC97 C	Industrie laitiére2 (lingette)	4	+	18,1	+	+	Ent.sakazakii	+	+	17,8	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC98 C	Industrie laitiére 3 (lingette)	4	+	21,9	+	+	Ent.sakazakii	+	+	17,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC99 C	Industrie laitiére 4 (lingette)	4	+	22,1	+	+	Ent.sakazakii	+	+	16,3	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC100 C	Industrie laitiére 5 (lingette)	4	+	33,8	+	+	Ent.sakazakii	+	+	15,3	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC101 C	Industrie laitiére 6 (lingette)	4	+	27,0	+	+	Ent.sakazakii	+	+	18,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC102 C	Industrie laitiére 7 (lingette)	4	+	31,9	+	+	Ent.sakazakii	+	+	22,9	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC103 C	Industrie laitiére 8 (lingette)	4	+	20,6	+	+	Ent.sakazakii	+	+	18,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC104 C	Industrie laitiére 9 (lingette)	2	+	34,2	+	+	Ent.sakazakii	+	+	28,7	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC105C	Industrie laitiére 10 (lingette)	2	+	27,5	+	+	Ent.sakazakii	+	+	24,3	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC106C	Industrie laitiére 11 (lingette)	2	+	19,0	+	+	Ent.sakazakii	+	+	17,0	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=
AC107C	Industrie laitiére 12 (lingette)	2	+	26,7	+	+	Ent.sakazakii	+	+	24,4	+	+	Ent.sakazakii	+	+	Ent.sakazakii	+	=	=

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP

Echantillons d'environnements (hors échantillons de surface) - Echantillons négatifs

n° echant	Produit	Taux d'inocu-lation (UFC / 25g)	MA: ADIAFOOD Cronobacter						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA
			PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final	
					Chrom ID sakazakii	ESIA	Galerie					
AC318	Poussières industrie laitiére		-					-	0		-	=
AC319	Poussières Salle A20		-					-	0		-	=
AC321	poussière industrie laitiére		-					-	-		-	=
AC322	Poussière industrie laitiére magasin		-					-	0		-	=
AC329	Poussière 1 (industrie laitiére)		-					-	-		-	=
AC331	Filtre 1 aspirateur industrie laitiére		-					-	0		-	=
AC332	Filtre 2 aspirateur industrie laitiére		-					-	-		-	=
AC333	Filtre 3 aspirateur industrie laitiére		-					-	-		-	=
AC334	Filtre 4 aspirateur industrie laitiére		-					-	-		-	=
AC366	Poussiére industrie laitiére		-					-	-		-	=
AC325	Eau de process 10A		-					-	-		-	=
AC326	Eau de process 13A		-					-	-		-	=
AC327	Eau de process 14A		-					-	0		-	=
AC328	Eau de process 15A		-					-	0		-	=
AC349	Eau de process Gallia Calisma		- **					-	0		-	=
AC350	Eau de process Carrefour Baby		-					-	0		-	=
AC351	Eau de process Modilac Digest 1		-					-	0		-	=
AC 352	Eau de process EA4341		-					-	0		-	=
AC353	Eau de process Sypac M80773		-					-	0		-	=
AC354	Eau de process LM6477		-					-	0		-	=

AES CHEMUNEX - ADIAFOOD CRONOBACTER SPP

Echantillons d'environnements (hors échantillons de surface) - Echantillons positifs

n° echant	Produit	Taux d'inocu-lation (UFC / 25g)	MA: ADIAFOOD Cronobacter						MR: ISO/TS 22964 (2006)(*)			Concordance MR /MA
			PCR	Ct	Confirmation			Résultat Final	ESIA	Galerie	Résultat final	
					Chrom ID sakazakii	ESIA	Galerie					
AC323	Poussière 1 atomiseur industrie laitiére	NC	+	28	+	+		+	+	Ent.sakazakii	+	=
AC324	Poussière 2 atomiseur industrie laitiére	NC	+	29,2	+	+		+	+	Ent.sakazakii	+	=
AC330	Poussière 2 industrie laitiére	NC	+	33,2	+	+		+	+	Ent.sakazakii	+	=
AC335	Poussière atomiseur laiterie	3	+	26,9	+	+		+	+	Ent.sakazakii	+	=
AC340	Poussière étage atomiseur industrie laitiére	3	+	29,5	+	+		+	+	Ent.sakazakii	+	=
AC341	Poussière industrie laitiére magasin	6	+ **	30,1	+	+		+	+	Ent.sakazakii	+	=
AC342	Filtre 1 aspirateur industrie laitiére	6	+	19	+	+		+	+	Ent.sakazakii	+	=
AC343	Filtre 2 aspirateur industrie laitiére	6	+	19,8	+	+		+	+	Ent.sakazakii	+	=
AC344	Filtre 3 aspirateur industrie laitiére	6	+	19,1	+	+		+	+	Ent.sakazakii	+	=
AC345	Filtre 4 aspirateur industrie laitiére	6	+	18,4	+	+		+	+	Ent.sakazakii	+	=
AC346	Poussières et filtre industrie laitiére	NC	+	27	+	+		+	+	Ent.sakazakii	+	=
AC347	Poussières aspirateur laiterie	NC	+	28,5	+	+		+	+	Ent.sakazakii	+	=
AC355	Poussières 2 industrie laitiére	4	+	30,1	+	+		+	+	Ent.sakazakii	+	=
AC356	Poussières aspirateur laiterie	4	+	25,9	+	+		+	+	Ent.sakazakii	+	=
AC361	Poussière aspirateur laiterie	5	+	27,5	+	+		+	+	Ent.sakazakii	+	=
AC336	Eau de process 10A	3	+	25,5	+	+		+	+	Ent.sakazakii	+	=
AC337	Eau de process 13A	3	+	17,8	+	+		+	+	Ent.sakazakii	+	=
AC338	Eau de process 14A	3	+	18,1	+	+		+	+	Ent.sakazakii	+	=
AC357	Eau de process chaine de prod 1	6	+	18,8	+	+		+	+	Ent.sakazakii	+	=
AC358	Eau de process chaine de prod 2	6	+	18,2	+	+		+	+	Ent.sakazakii	+	=
AC359	Eau de process chaine de prod 3	10	+	18,2	+	+		+	+	Ent.sakazakii	+	=
AC360	Eau de process chaine de prod 4	10	+	18,2	+	+		+	+	Ent.sakazakii	+	=
AC362	Eau de process chaine de prod 5	5	+	18	+	+		+	+	Ent.sakazakii	+	=
AC363	Eau de process chaine de prod 6	5	+	17,9	+	+		+	+	Ent.sakazakii	+	=
AC364	Eau de process chaine de prod 7	5	+	17,3	+	+		+	+	Ent.sakazakii	+	=

LOW CONFIDENCE LEVELS

Catégorie	PA	NA	ND	PD	N	Exactitude relative AC (%)	LCI	N+	Sensibilité relative SE (%)	LCI	N-	Spécificité relative SP (%)	LCI
Produits laitiers	26	32	1	3	62	93,5%	89%	27	96,3%	92%	35	91,4%	86%
Divers	31	32	1	0	64	98,4%	96%	32	96,9%	92%	32	100,0%	98%
Echantillons d'environnement (échantillons de surfaces)	30	33	0	1	64	98,4%	96%	30	100,0%	98%	36	97,2%	96%
Echantillons d'environnement (poussières, eaux de process…)	25	20	0	0	45	100,0%	97%	25	100,0%	98%	20	100,0%	98%
Tous produits	112	117	2	4	235	97,4%	93%	114	98,2%	96%	121	96,7%	93%

Legende:

LCI: Limites de confiance inférieure
PA: accord positif
NA: accord négatif
ND: déviation négative
PD: déviation positive
N=PA+NA+PD+ND
N+=PA+ND
N-=NA+PD

APPENDIX 5

RELATIVE DETECTION LEVEL

RAW RESULTS

AES CHEMUNEX - ADIAFOOD CRONOBACTER - NDR Produits laitiers (lait en poudre)

Flore totale: de 130 UFC/g

		MA: ADIAFOOD Cronobacter				MR: ISO/TS 22964 (2006)(*)		Concordance (MA/MR)
N°	Contamination en UFC/50g (taux réel)	Résultat PCR	CT	Confirmation	Résultat final	ESIA	Résultat final	
0A	0	-			-	-	-	MA: 0/6
0B		-			-	-	-	
0C		-			-	-	-	
0D		-			-	-	-	
0E		-			-	-	-	MR: 0/6
0F		-			-	-	-	
0,2A	0,3	-			-	-	-	MA: 1/6
0,2B		-			-	-	-	
0,2C		-			-	-	-	
0,2D		-			-	+	+	
0,2E		-			-	-	-	MR: 1/6
0,2F		+	24,1	+	+	-	-	
0,5A	0,6	+	20	+	+	-	-	MA: 2/6
0,5B		-			-	-	-	
0,5C		+	34,4	+	+	-	-	
0,5D		-			-	+	+	
0,5E		-			-	-	-	MR: 2/6
0,5F		-			-	+	+	
1A	1	+	25,9	+	+	+	+	MA: 4/6
1B		-			-	+	+	
1C		+	28	+	+	+	+	
1D		+	24,4	+	+	-	-	
1E		-			-	-	-	MR: 4/6
1F		+	25	+	+	+	+	
2A	2	+	27,7	+	+	+	+	MA: 6/6
2B		+	27,8	+	+	+	+	
2C		+	27,5	+	+	+	+	
2D		+	25,5	+	+	+	+	
2E		+	24,1	+	+	+	+	MR: 6/6
2F		+	25,1	+	+	+	+	

AES CHEMUNEX - ADIAFOOD CRONOBACTER - NDR Produits divers (Lait de soja)

Flore totale: <10 UFC/g

		MA: ADIAFOOD Cronobacter				MR: ISO/TS 22964 (2006)(*)		Concordance (MA/MR)
N°	Contamination en UFC/50g (taux réel)	Résultat PCR	CT	Confirmation	Résultat final	ESIA	Résultat final	
0A	0	-			-	-	-	MA: 0/6
0B		-			-	-	-	
0C		-			-	-	-	
0D		-			-	-	-	
0E		-			-	-	-	MR: 0/6
0F		-			-	-	-	
0,2A	0,4	-			-	-	-	MA: 1//6
0,2B		-			-	-	-	
0,2C		+	34,6	+	+	+	+	
0,2D		-			-	-	-	
0,2E		-			-	-	-	MR: 1/6
0,2F		-			-	-	-	
0,75A	0,9	+	32,8	+	+	+	+	MA: 2/6
0,75B		-			-	-	-	
0,75C		-			-	-	-	
0,75D		+	33,6	+	+	-	-	
0,75E		-			-	-	-	MR: 1/6
0,75F		-			-	-	-	
1A	1,3	+	35,1	+	+	+	+	MA: 5/6
1B		+	35,3	+	+	+	+	
1C		-			-	+	+	
1D		+	35,2	+	+	-	-	
1E		+	35,1	+	+	+	+	MR: 5/6
1F		+	32,2	+	+	+	+	
5A	5	+	30,4	+	+	+	+	MA: 6/6
5B		+	30,8	+	+	+	+	
5C		+	31,7	+	+	+	+	
5D		+	32,9	+	+	+	+	
5E		+	34,7	+	+	+	+	MR: 6/6
5F		+	34,4	+	+	+	+	

AES CHEMUNEX - ADIAFOOD CRONOBACTER - NDR Echantillons d'environnement

Flore totale: 2,5 x10^4 UFC/g

		MA: ADIAFOOD Cronobacter				MR: ISO/TS 22964 (2006)(*)		Concordance (MA/MR)
N°	Contamination en UFC/50g (taux réel)	Résultat PCR	CT	Confirmation	Résultat final	ESIA	Résultat final	
0A	0	-			-	-	-	MA: 0/6
0B		-			-	-	-	
0C		-			-	-	-	
0D		-			-	-	-	
0E		-			-	-	-	MR: 0/6
0F		-			-	-	-	
0,5A	0,4	-			-	-	-	MA: 1/6
0,5B		-			-	-	-	
0,5C		+	34,6	+	+	+	+	
0,5D		-			-	-	-	
0,5E		-			-	-	-	MR: 1/6
0,5F		-			-	-	-	
0,75A	0,7	-			-	-	-	MA: 2/6
0,75B		-			-	-	-	
0,75C		-			-	-	-	
0,75D		+	33,6	+	+	+	+	
0,75E		-			-	-	-	MR: 2/6
0,75F		+	34,2	+	+	+	+	
1A	1,5	+	34,5	+	+	+	+	MA: 5/6
1B		+	34	+	+	+	+	
1C		+	32,7	+	+	+	+	
1D		-			-	+	+	
1E		+	33,9	+	+	+	+	MR: 6/6
1F		+	34,9	+	+	+	+	
5A	5	+	30,4	+	+	+	+	MA: 6/6
5B		+	30,8	+	+	+	+	
5C		+	31,7	+	+	+	+	
5D		+	32,9	+	+	+	+	
5E		+	34,7	+	+	+	+	MR: 6/6
5F		+	34,4	+	+	+	+	

APPENDIX 6 - SELECTIVITY - INCLUSIVITY
BPW + supplement, incubation 16h

souche cibles	Microorganisme	Origine	Taux d'inoculation (UFC/450 mL)	Résultat PCR	Ct	Conf MA
ENTB.3.1	*Cronobacter sakazakii subsp sakazakii*	Poudre de lait	22	+	18,0	+
ENTB.3.10	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	28	+	34,1	+
ENTB.3.11	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	24	+	30,9	+
ENTB.3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	3	+	24,6	+
ENTB.3.13	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	30	+	33,9	+
ENTB.3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	8	+	31,4	+
ENTB.3.15	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	14	+	22,0	+
ENTB.3.16	*Cronobacter spp*	Environnement industriel	36	+	17,2	+
ENTB.3.17	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	12	+	16,8	+
ENTB.3.19	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	13	+	29,5	+
ENTB.3.20	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	26	+	20,0	+
ENTB.3.21	*Cronobacter muytjensii*	CIP 103581	30	+	17,8	+
ENTB.3.22	*Cronobacter sakazakii*	CIP 103183	18	+	21,2	+
ENTB.3.23	*Cronobacter sakazakii*	CIP 104951	3	+	35,1	+
ENTB.3.24	*Cronobacter sakazakii*	CIP 104952	4	+	34,2	+
ENTB.3.25	*Cronobacter sakazakii subsp sakazakii*	Environement industriel	18	+	32,4	+
ENTB.3.26	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	10	+	21,3	+
ENTB.3.27	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	25	+	20,2	+
ENTB.3.28	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	26	+	21,3	+
ENTB.3.29	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	26	+	27,4	+
ENTB.3.3	*Cronobacter sakazakii*	CIP 57.33	25	+	22,7	+
ENTB.3.30	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	15	+	21,3	+
ENTB.3.31	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	23	+	35,1	+
ENTB.3.32	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	9	+	20,4	+
ENTB.3.33	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	30	+	17,9	+
ENTB.3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	23	+	25,8	+
ENTB.3.35	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	14	+	32,2	+
ENTB.3.36	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	12	+	21,9	+
ENTB.3.37	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	21	+	32,9	+
ENTB.3.38	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	8	+	27,6	+
ENTB.3.39	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	19	+	33,9	+
ENTB.3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pasteur de Lille	16	+	18,2	+
ENTB.3.40	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	19	+	23,1	+
ENTB.3.41	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	21	+	34,8	+
ENTB.3.42	*Cronobacter sakazakii subsp sakazakii*	Ennvironement industriel	23	+	33,3	+
ENTB.3.43	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	34	+	29,6	+
ENTB.3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	21	+	20,7	+
ENTB.3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	12	+	28,2	+
ENTB.3.46	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	23	+	19,2	+
ENTB.3.47	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	8	+	22,9	+
ENTB.3.48	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	8	+	32,6	+
ENTB.3.5	*Cronobacter sakazakii subsp sakazakii*	Institut Pasteur de Lille	26	+	34,9	+
ENTB.3.51	*Cronobacter turicensis*	ADRIA developpement	6	+	18,8	+
ENTB.3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	10	+	17,2	+
ENTB.3.53	*Cronobacter turicensis*	DSM18703	24	+	21,5	+
ENTB.3.54	*Cronobacter dublinensis subsp dublinensis*	DSM18705	17	+	20,7	+
ENTB.3.55	*Cronobacter dublinensis subsp lausannensis*	DSM18706	6	+	21,2	+
ENTB.3.56	*Cronobacter dublinensis subsp lactaridi*	DSM19707	20	+	21,1	+
ENTB.3.57	*Cronobacter muytjensii*	DSM 21870	24	+	17,6	+
ENTB.3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	22	+	20,4	+

APPENDIX 6 - SELECTIVITY - INCLUSIVITY
BPW, incubation 8h

souche cibles	Microorganisme	Origine	Taux d'inoculation (UFC/450 mL)	Résultat PCR	Ct	Conf MA
ENTB.3.1	*Cronobacter sakazakii subsp sakazakii*	Poudre de lait	31	+	34,0	+
ENTB.3.10	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	17	+	34,5	+
ENTB.3.11	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	16	+	35,0	+
ENTB.3.12	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	45	+	34,8	+
ENTB.3.13	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	16	+	32,5	+
ENTB.3.14	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	13	+	33,6	+
ENTB.3.15	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	13	+	34,8	+
ENTB.3.16	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	7	+	33,9	+
ENTB.3.17	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	10	+	29,3	+
ENTB.3.19	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	17	+	34,2	+
ENTB.3.20	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	21	+	34,7	+
ENTB.3.21	*Cronobacter muytjensii*	CIP 103581	24	+	34,0	+
ENTB.3.22	*Cronobacter sakazakii*	CIP 103183	32	+	33,2	+
ENTB.3.23	*Cronobacter sakazaki*	CIP 104951	13	+	33,5	+
ENTB.3.24	*Cronobacter sakazakii*	CIP 104952	7	+	34,5	+
ENTB.3.25	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	9	+	34,5	+
ENTB.3.26	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	30	+	34,9	+
ENTB.3.27	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	26	+	34,4	+
ENTB.3.28	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	16	+	35,2	+
ENTB.3.29	*Cronobacter sakazakii*	Environnement industrie laitière	45	+	34,1	+
ENTB.3.3	*Cronobacter sakazakii*	Poudre de lait	14	+	34,4	+
ENTB.3.30	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	22	+	32,9	+
ENTB.3.31	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	9	+	33,7	+
ENTB.3.32	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	19	+	34,4	+
ENTB.3.33	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	13	+	32,7	+
ENTB.3.34	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	17	+	35,2	+
ENTB.3.35	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	24	+	34,8	+
ENTB.3.36	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	16	+	34,9	+
ENTB.3.37	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	28	+	33,9	+
ENTB.3.38	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	13	+	35,0	+
ENTB.3.39	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	8	+	34,6	+
ENTB.3.4	*Cronobacter sakazakii subsp sakazakii*	Institut Pasteur de Lille	45	+	34,5	+
ENTB.3.40	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	18	+	35,0	+
ENTB.3.41	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	12	+	33,7	+
ENTB.3.42	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	8	+	33,0	+
ENTB.3.43	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	21	+	33,9	+
ENTB.3.44	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	11	+	34,5	+
ENTB.3.45	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	25	+	34,3	+
ENTB.3.46	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	16	+	35,1	+
ENTB.3.47	*Cronobacter sakazakii subsp malonaticus*	Environnement industriel	5	+	29,9	+
ENTB.3.48	*Cronobacter sakazakii subsp sakazakii*	Environnement industriel	33	+	34,3	+
ENTB.3.5	*Cronobacter sakazakii subsp sakazakii*	Institut Pasteur de Lille	9	+	34,4	+
ENTB.3.51	*Cronobacter turicensis*	ADRIA developpement	33	+	35,1	+
ENTB.3.52	*Cronobacter spp*	Ingrédient poudre de lait: blé chauffé	11	+	34,3	+
ENTB.5.53	*Cronobacter turicensis*	DSM18703	14	+	31,0	+
ENTB.3.54	*Cronobacter dublinensis subsp dublinensis*	DSM18705	22	+	33,1	+
ENTB.3.55	*Cronobacter dublinensis subsp lausannensis*	DSM18706	26	+	33,1	+
ENTB.3.56	*Cronobacter dublinensis subsp lactaridi*	DSM19707	18	+	33,6	+
ENTB.3.57	*Cronobacter muytjensii*	DSM 21870	21	+	31,1	+
ENTB.3.6	*Cronobacter sakazakii subsp sakazakii*	Environnement	7	+	35,0	+

APPENDIX 6 - SELECTIVITY - EXCLUSIVITY

Codes souche	Nom de la souche	Origine	résultat méthode alternative		
			Résultat PCR	Ct	Confirmation ESIA ou Chrom ID Sakazaki
ENTB.1.1	*Enterobacter aerogenes*	industrie laitière	-		
ENTB.1.2	*Enterobacter aerogenes*	CIP 60.86 T	-		
ENTB.2.1	*Enterobacter cloacae*	Eaux usagées	-		
ENTC.2.1	*Enterococcus faecium*	industrie laitière	-		
ESC.1.3	*Escherichia coli*	industrie laitière	-		
ESC.1.5	*Escherichia coli*	Camembert	-		
HAF.1.1	*Hafnia alvei*	taboulé	-		
HAN.1.1	*Hansenula anomala*	industrie laitière	-		
LACB.1.1	*Lactobacillus casei*	produit laitier	-		
STA.2.1	*Staphylococcus epidermidis*	produit laitier	-		
PSE.1.2	*Pseudomonas aeruginosa*	omelette gruyère	-		
PSE.2.2	*Pseudomonas fluorescens*	CIP102127	-		
SAL.1.98	*Salmonella enterica Newport*	Fromage au lait cru	-		
SAL.1.163	*Salmonella enterica Infantis*	Lait (alim. humaine)	-		
SER.1.1	*Serratia ficaria*	CIP 79.23	-		
SER.2.1	*Serratia fonticola*	CIP 103580	-		
SHI.1.1	*Shigella flexneri*	CIP 82.48T	-		
ESC.2.1	*Escherichia hermanii*	CIP 103176	-		
CIT.1.1	*Citrobacter freundii*	CIP 53.62	-		
KLE.2.1	*Klebsiella pneumoniae*	pâtisserie	-		
KLE.1.1	*Klebsiella oxytoca*	salade soja	-		
CIT.2.1	*Citrobacter koseri*	CIP 72.11	-		
ENTB.4.1	*Enterobacter hormaechei*	CIP 104956	-		
ENTB.5.1	*Enterobacter amnigenus*	CIP 104982	-		
ENTB.9.1	*Enterobacter asburiae*	CIP 66.35	-		
ENTB.7.1	*Enterobacter nimipressuralis*	CIP 105047	-		
ENTB.8.1	*Enterobacter pyrinus*	CIP 104019	-		
SAL.1.121	*Salmonella enterica Salamae*	Lait cru	-		
SHI.2.1	*Shigella sonnei*	ATCC 9290	-		
SAL.1.6	*Salmonella enterica Arizonae*	Saucisson sec	-		

Appendix 7
Total flora enumeration

Laboratoire	Résultats du dénombrement (UFC/g)
A	<10
B	<10
C	<10
D	17000
E	18
F	<40
G	<10
H	10
I	<10
J	<10000
Expert	<10

Appendix 7
Expert laboratory results

A=absence, P=présence, +=test positif, -=test négatif, /=test non réalisé

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
ISHA1	+	+	P	**ISHA1**	+	+	P
ISHA2	-	/	A	**ISHA2**	-	/	A
ISHA3	+	+	P	**ISHA3**	+	+	P
ISHA4	+	+	P	**ISHA4**	+	+	P
ISHA5	+	+	P	**ISHA5**	+	+	P
ISHA6	-	/	A	**ISHA6**	-	/	A
ISHA7	-	/	A	**ISHA7**	-	/	A
ISHA8	+	+	P	**ISHA8**	+	+	P
ISHA9	+	+	P	**ISHA9**	+	+	P
ISHA10	-	/	A	**ISHA10**	-	/	A
ISHA11	+	+	P	**ISHA11**	+	+	P
ISHA12	+	+	P	**ISHA12**	+	+	P
ISHA13	-	/	A	**ISHA13**	-	/	A
ISHA14	+	+	P	**ISHA14**	+	+	P
ISHA15	+	+	P	**ISHA15**	+	+	P
ISHA16	-	/	A	**ISHA16**	-	/	A
ISHA17	+	+	P	**ISHA17**	+	+	P
ISHA18	+	+	P	**ISHA18**	+	+	P
ISHA19	+	+	P	**ISHA19**	+	+	P
ISHA20	+	+	P	**ISHA20**	+	+	P
ISHA21	-	/	A	**ISHA21**	-	/	A
ISHA22	+	+	P	**ISHA22**	+	+	P
ISHA23	+	+	P	**ISHA23**	+	+	P
ISHA24	-	/	A	**ISHA24**	-	/	A

Appendix 7
Participating laboratories results

A=absence, P=présence, +=test positif, -=test négatif, /=test non réalisé

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
A1	+	+	P	**A1**	+	+	P
A2	-	/	A	**A2**	-	/	A
A3	+	+	P	**A3**	+	+	P
A4	+	+	P	**A4**	+	+	P
A5	+	+	P	**A5**	+	+	P
A6	-	/	A	**A6**	-	/	A
A7	-	/	A	**A7**	-	/	A
A8	+	+	P	**A8**	+	+	P
A9	+	+	P	**A9**	+	+	P
A10	-	/	A	**A10**	-	/	A
A11	+	+	P	**A11**	+	+	P
A12	+	+	P	**A12**	+	+	P
A13	-	/	A	**A13**	-	/	A
A14	+	+	P	**A14**	+	+	P
A15	+	+	P	**A15**	+	+	P
A16	-	/	A	**A16**	-	/	A
A17	+	+	P	**A17**	+	+	P
A18	+	+	P	**A18**	+	+	P
A19	+	+	P	**A19**	+	+	P
A20	+	+	P	**A20**	+	+	P
A21	-	/	A	**A21**	-	/	A
A22	+	+	P	**A22**	+	+	P
A23	+	+	P	**A23**	+	+	P
A24	-	/	A	**A24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
B1	+	+	P	**B1**	+	+	P
B2	-	/	A	**B2**	-	/	A
B3	+	+	P	**B3**	+	+	P
B4	+	+	P	**B4**	+	+	P
B5	+	+	P	**B5**	+	+	P
B6	-	/	A	**B6**	-	/	A
B7	-	/	A	**B7**	-	/	A
B8	+	+	P	**B8**	+	+	P
B9	+	+	P	**B9**	+	+	P
B10	-	/	A	**B10**	-	/	A
B11	+	+	P	**B11**	+	+	P
B12	+	+	P	**B12**	+	+	P
B13	-	/	A	**B13**	-	/	A
B14	+	+	P	**B14**	+	+	P
B15	+	+	P	**B15**	+	+	P
B16	-	/	A	**B16**	-	/	A
B17	+	+	P	**B17**	+	+	P
B18	+	+	P	**B18**	+	+	P
B19	+	+	P	**B19**	+	+	P
B20	-	-	A	**B20**	+	+	P
B21	-	/	A	**B21**	-	/	A
B22	+	+	P	**B22**	+	+	P
B23	+	+	P	**B23**	+	+	P
B24	-	/	A	**B24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
C1	+	+	P	**C1**	+	+	P
C2	-	/	A	**C2**	-	/	A
C3	+	+	P	**C3**	+	+	P
C4	+	+	P	**C4**	+	+	P
C5	+	+	P	**C5**	+	+	P
C6	-	/	A	**C6**	-	/	A
C7	-	/	A	**C7**	-	/	A
C8	+	+	P	**C8**	+	+	P
C9	+	+	P	**C9**	+	+	P
C10	-	/	A	**C10**	-	/	A
C11	+	+	P	**C11**	+	+	P
C12	+	+	P	**C12**	+	+	P
C13	-	/	A	**C13**	-	/	A
C14	+	+	P	**C14**	+	+	P
C15	+	+	P	**C15**	+	+	P
C16	-	/	A	**C16**	-	/	A
C17	+	+	P	**C17**	+	+	P
C18	+	+	P	**C18**	+	+	P
C19	+	+	P	**C19**	+	+	P
C20	+	+	P	**C20**	+	+	P
C21	-	/	A	**C21**	-	/	A
C22	+	+	P	**C22**	+	+	P
C23	+	+	P	**C23**	+	+	P
C24	-	/	A	**C24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
D1	+	+	P	**D1**	+	+	P
D2	-	/	A	**D2**	-	/	A
D3	+	+	P	**D3**	+	+	P
D4	+	+	P	**D4**	+	+	P
D5	+	+	P	**D5**	+	+	P
D6	-	/	A	**D6**	-	/	A
D7	-	/	A	**D7**	-	/	A
D8	+	+	P	**D8**	+	+	P
D9	+	+	P	**D9**	+	+	P
D10	-	/	A	**D10**	-	/	A
D11	+	+	P	**D11**	+	+	P
D12	+	+	P	**D12**	+	+	P
D13	-	/	A	**D13**	-	/	A
D14	+	+	P	**D14**	+	+	P
D15	+	+	P	**D15**	+	+	P
D16	-	/	A	**D16**	-	/	A
D17	+	+	P	**D17**	+	+	P
D18	+	+	P	**D18**	+	+	P
D19	+	+	P	**D19**	+	+	P
D20	+	+	P	**D20**	+	+	P
D21	-	/	A	**D21**	-	/	A
D22	+	+	P	**D22**	+	+	P
D23	+	+	P	**D23**	+	+	P
D24	-	/	A	**D24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
E1	+	+	P	**E1**	+	+	P
E2	-	/	A	**E2**	-	/	A
E3	+	+	P	**E3**	+	+	P
E4	+	+	P	**E4**	+	+	P
E5	+	+	P	**E5**	+	+	P
E6	-	/	A	**E6**	-	/	A
E7	-	/	A	**E7**	-	/	A
E8	+	+	P	**E8**	+	+	P
E9	+	+	P	**E9**	+	+	P
E10	-	/	A	**E10**	-	/	A
E11	+	+	P	**E11**	+	+	P
E12	+	+	P	**E12**	+	+	P
E13	-	/	A	**E13**	-	/	A
E14	+	+	P	**E14**	+	+	P
E15	-	/	A	**E15**	-	/	A
E16	-	/	A	**E16**	-	/	A
E17	+	+	P	**E17**	+	+	P
E18	+	+	P	**E18**	+	+	P
E19	+	+	P	**E19**	+	+	P
E20	+	+	P	**E20**	+	+	P
E21	-	/	A	**E21**	-	/	A
E22	+	+	P	**E22**	+	+	P
E23	+	+	P	**E23**	+	+	P
E24	-	/	A	**E24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
F1	+	+	P	**F1**	+	+	P
F2	-	/	A	**F2**	-	/	A
F3	+	+	P	**F3**	+	+	P
F4	+	+	P	**F4**	+	+	P
F5	+	+	P	**F5**	+	+	P
F6	-	/	A	**F6**	-	/	A
F7	-	/	A	**F7**	-	/	A
F8	+	+	P	**F8**	+	+	P
F9	+	+	P	**F9**	+	+	P
F10	-	/	A	**F10**	-	/	A
F11	+	+	P	**F11**	+	+	P
F12	+	+	P	**F12**	+	+	P
F13	-	/	A	**F13**	-	/	A
F14	+	+	P	**F14**	+	+	P
F15	+	+	P	**F15**	+	+	P
F16	-	/	A	**F16**	-	/	A
F17	+	+	P	**F17**	+	+	P
F18	+	+	P	**F18**	+	+	P
F19	+	+	P	**F19**	+	+	P
F20	+	+	P	**F20**	+	+	P
F21	-	/	A	**F21**	-	/	A
F22	+	+	P	**F22**	+	+	P
F23	+	+	P	**F23**	+	+	P
F24	-	/	A	**F24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
G1	+	+	P	**G1**	+	+	P
G2	-	/	A	**G2**	-	/	A
G3	+	+	P	**G3**	+	+	P
G4	+	+	P	**G4**	+	+	P
G5	+	+	P	**G5**	+	+	P
G6	-	/	A	**G6**	-	/	A
G7	-	/	A	**G7**	-	/	A
G8	+	+	P	**G8**	+	+	P
G9	+	+	P	**G9**	+	+	P
G10	-	/	A	**G10**	-	/	A
G11	+	+	P	**G11**	+	+	P
G12	+	+	P	**G12**	+	+	P
G13	-	/	A	**G13**	-	/	A
G14	+	+	P	**G14**	+	+	P
G15	+	+	P	**G15**	+	+	P
G16	-	/	A	**G16**	-	/	A
G17	+	+	P	**G17**	+	+	P
G18	+	+	P	**G18**	+	+	P
G19	+	+	P	**G19**	+	+	P
G20	+	+	P	**G20**	+	+	P
G21	-	/	A	**G21**	-	/	A
G22	+	+	P	**G22**	+	+	P
G23	+	+	P	**G23**	+	+	P
G24	-	/	A	**G24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
H1	+	+	P	**H1**	+	+	P
H2	-	/	A	**H2**	-	/	A
H3	+	+	P	**H3**	+	+	P
H4	+	+	P	**H4**	+	+	P
H5	+	+	P	**H5**	+	+	P
H6	-	/	A	**H6**	-	/	A
H7	-	/	A	**H7**	-	/	A
H8	+	+	P	**H8**	+	+	P
H9	+	+	P	**H9**	+	+	P
H10	-	-	A	**H10**	+	+	P
H11	+	+	P	**H11**	+	+	P
H12	+	+	P	**H12**	+	+	P
H13	-	/	A	**H13**	-	/	A
H14	+	+	P	**H14**	+	+	P
H15	+	+	P	**H15**	+	+	P
H16	-	/	A	**H16**	-	/	A
H17	+	+	P	**H17**	+	+	P
H18	+	+	P	**H18**	+	+	P
H19	+	+	P	**H19**	+	+	P
H20	+	+	P	**H20**	+	+	P
H21	-	/	A	**H21**	-	/	A
H22	+	+	P	**H22**	+	+	P
H23	+	+	P	**H23**	+	+	P
H24	-	/	A	**H24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
I1	+	+	P	**I1**	+	+	P
I2	-	/	A	**I2**	-	/	A
I3	+	+	P	**I3**	+	+	P
I4	+	+	P	**I4**	+	+	P
I5	+	+	P	**I5**	+	+	P
I6	-	/	A	**I6**	-	/	A
I7	-	/	A	**I7**	-	/	A
I8	+	+	P	**I8**	+	+	P
I9	+	+	P	**I9**	+	+	P
I10	-	/	A	**I10**	-	/	A
I11	+	+	P	**I11**	+	+	P
I12	+	+	P	**I12**	-	-	A
I13	-	/	A	**I13**	-	/	A
I14	+	+	P	**I14**	+	+	P
I15	+	+	P	**I15**	+	+	P
I16	-	/	A	**I16**	-	/	A
I17	+	+	P	**I17**	+	+	P
I18	+	+	P	**I18**	-	-	A
I19	+	+	P	**I19**	+	+	P
I20	+	+	P	**I20**	+	+	P
I21	-	/	A	**I21**	-	/	A
I22	+	+	P	**I22**	+	+	P
I23	+	+	P	**I23**	+	+	P
I24	-	/	A	**I24**	-	/	A

Code	Méthode Alternative			Code	Méthode de Référence		
	ADIAFOOD Cronobacter				ISO/TS 22964 (2006)		
	ADIAFOOD	Confir-mation	Résultat Final		ESIA	Confir-mation	Résultat final
J1	+	+	P	**J1**	+	+	P
J2	-	/	A	**J2**	-	/	A
J3	+	+	P	**J3**	+	+	P
J4	+	+	P	**J4**	+	+	P
J5	+	+	P	**J5**	+	+	P
J6	-	/	A	**J6**	-	/	A
J7	-	/	A	**J7**	-	/	A
J8	+	+	P	**J8**	+	+	P
J9	+	+	P	**J9**	+	+	P
J10	-	/	A	**J10**	-	/	A
J11	+	+	P	**J11**	+	+	P
J12	+	+	P	**J12**	+	+	P
J13	-	/	A	**J13**	-	/	A
J14	+	+	P	**J14**	+	+	P
J15	+	+	P	**J15**	+	+	P
J16	-	/	A	**J16**	-	/	A
J17	+	+	P	**J17**	+	+	P
J18	+	+	P	**J18**	+	+	P
J19	+	+	P	**J19**	+	+	P
J20	+	+	P	**J20**	+	+	P
J21	-	/	A	**J21**	-	/	A
J22	+	+	P	**J22**	+	+	P
J23	+	+	P	**J23**	+	+	P
J24	-	-	A	**J24**	+	+	P

Appendix 8 - Accordance calculations

Méthode alternative Nombre de réplicats: 8

Niveau L0

Laboratoire	Nombre de positifs	Probabilité de positifs	Probabilité de paires de positifs	Probabilité de négatifs	Probabilité de paires de négatifs	Probabilité de paires de résultats identiques
A	0	0,000	0,000	1,000	1,000	1,000
B	0	0,000	0,000	1,000	1,000	1,000
C	0	0,000	0,000	1,000	1,000	1,000
D	0	0,000	0,000	1,000	1,000	1,000
E	0	0,000	0,000	1,000	1,000	1,000
F	0	0,000	0,000	1,000	1,000	1,000
G	0	0,000	0,000	1,000	1,000	1,000
H	0	0,000	0,000	1,000	1,000	1,000
I	0	0,000	0,000	1,000	1,000	1,000
J	0	0,000	0,000	1,000	1,000	1,000
Moyenne						100,0%

Niveau L1

Laboratoire	Nombre de positifs	Probabilité de positifs	Probabilité de paires de positifs	Probabilité de négatifs	Probabilité de paires de négatifs	Probabilité de paires de résultats identiques
A	8	1,000	1,000	0,000	0,000	1,000
B	7	0,875	0,766	0,125	0,016	0,781
C	8	1,000	1,000	0,000	0,000	1,000
D	8	1,000	1,000	0,000	0,000	1,000
E	7	0,875	0,766	0,125	0,016	0,781
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	8	1,000	1,000	0,000	0,000	1,000
I	8	1,000	1,000	0,000	0,000	1,000
J	8	1,000	1,000	0,000	0,000	1,000
Moyenne						95,6%

Niveau L2

Laboratoire	Nombre de positifs	Probabilité de positifs	Probabilité de paires de positifs	Probabilité de négatifs	Probabilité de paires de négatifs	Probabilité de paires de résultats identiques
A	8	1,000	1,000	0,000	0,000	1,000
B	8	1,000	1,000	0,000	0,000	1,000
C	8	1,000	1,000	0,000	0,000	1,000
D	8	1,000	1,000	0,000	0,000	1,000
E	8	1,000	1,000	0,000	0,000	1,000
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	8	1,000	1,000	0,000	0,000	1,000
I	8	1,000	1,000	0,000	0,000	1,000
J	8	1,000	1,000	0,000	0,000	1,000
Moyenne						100,0%

Méthode de référence Nombre de réplicats: 8

Niveau L0

Laboratoire	Nombre de positifs	Probabilité de positifs	Probabilité de paires de positifs	Probabilité de négatifs	Probabilité de paires de négatifs	Probabilité de paires de résultats identiques
A	0	0,000	0,000	1,000	1,000	1,000
B	0	0,000	0,000	1,000	1,000	1,000
C	0	0,000	0,000	1,000	1,000	1,000
D	0	0,000	0,000	1,000	1,000	1,000
E	0	0,000	0,000	1,000	1,000	1,000
F	0	0,000	0,000	1,000	1,000	1,000
G	0	0,000	0,000	1,000	1,000	1,000
H	1	0,125	0,016	0,875	0,766	0,781
I	0	0,000	0,000	1,000	1,000	1,000
J	1	0,125	0,016	0,875	0,766	0,781
Moyenne						95,6%

Niveau L1

Laboratoire	Nombre de positifs	Probabilité de positifs	Probabilité de paires de positifs	Probabilité de négatifs	Probabilité de paires de négatifs	Probabilité de paires de résultats identiques
A	8	1,000	1,000	0,000	0,000	1,000
B	8	1,000	1,000	0,000	0,000	1,000
C	8	1,000	1,000	0,000	0,000	1,000
D	8	1,000	1,000	0,000	0,000	1,000
E	7	0,875	0,766	0,125	0,016	0,781
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	8	1,000	1,000	0,000	0,000	1,000
I	6	0,750	0,563	0,250	0,063	0,625
J	8	1,000	1,000	0,000	0,000	1,000
Moyenne						94,1%

Niveau L2

Laboratoire	Nombre de positifs	Probabilité de positifs	Probabilité de paires de positifs	Probabilité de négatifs	Probabilité de paires de négatifs	Probabilité de paires de résultats identiques
A	8	1,000	1,000	0,000	0,000	1,000
B	8	1,000	1,000	0,000	0,000	1,000
C	8	1,000	1,000	0,000	0,000	1,000
D	8	1,000	1,000	0,000	0,000	1,000
E	8	1,000	1,000	0,000	0,000	1,000
F	8	1,000	1,000	0,000	0,000	1,000
G	8	1,000	1,000	0,000	0,000	1,000
H	8	1,000	1,000	0,000	0,000	1,000
I	8	1,000	1,000	0,000	0,000	1,000
J	8	1,000	1,000	0,000	0,000	1,000
Moyenne						100,0%

Appendix 9 - Concordance calculations

Méthode alternative

Niveau L0

Laboratoire	Nombre de négatifs	Paires interlaboratoires avec le même résultat	Nombre total de paires interlaboratoires
A	8	576	576
B	8	576	576
C	8	576	576
D	8	576	576
E	8	576	576
F	8	576	576
G	8	576	576
H	8	576	576
I	8	576	576
J	8	576	576
Total		5760	5760
Concordance		100,0%	

Niveau L1

Laboratoire	Nombre de positifs	Paires interlaboratoires avec le même résultat	Nombre total de paires interlaboratoires
A	8	560	576
B	7	498	576
C	8	560	576
D	8	560	576
E	7	498	576
F	8	560	576
G	8	560	576
H	8	560	576
I	8	560	576
J	8	560	576
Total		5476	5760
Concordance		95,1%	

Niveau L2

Laboratoire	Nombre de positifs	Paires interlaboratoires avec le même résultat	Nombre total de paires interlaboratoires
A	8	576	576
B	8	576	576
C	8	576	576
D	8	576	576
E	8	576	576
F	8	576	576
G	8	576	576
H	8	576	576
I	8	576	576
J	8	576	576
Total		5760	5760
Concordance		100,0%	

Méthode de référence

Niveau L0

Laboratoire	Nombre de négatifs	Paires interlaboratoires avec le même résultat	Nombre total de paires interlaboratoires
A	8	560	576
B	8	560	576
C	8	560	576
D	8	560	576
E	8	560	576
F	8	560	576
G	8	560	576
H	7	498	576
I	8	560	576
J	7	498	576
Total		5476	5760
Concordance		95,1%	

Niveau L1

Laboratoire	Nombre de positifs	Paires interlaboratoires avec le même résultat	Nombre total de paires interlaboratoires
A	8	552	576
B	8	552	576
C	8	552	576
D	8	552	576
E	7	492	576
F	8	552	576
G	8	552	576
H	8	552	576
I	6	428	576
J	8	552	576
Total		5336	5760
Concordance		92,6%	

Niveau L2

Laboratoire	Nombre de positifs	Paires interlaboratoires avec le même résultat	Nombre total de paires interlaboratoires
A	8	576	576
B	8	576	576
C	8	576	576
D	8	576	576
E	8	576	576
F	8	576	576
G	8	576	576
H	8	576	576
I	8	576	576
J	8	576	576
Total		5760	5760
Concordance		100,0%	