

**NF VALIDATION**  
**Validation of alternative analytical methods**  
*Application in food microbiology*

**Summary report**

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

**TEMPO® EC**

(Certificate number: BIO 12/13 - 02/05)

**for the enumeration of  $\beta$  glucuronidase-positive *Escherichia coli* in  
all human food products and pet foods**

**Quantitative method**

<b>&gt; Expert Laboratory:</b>	<b>ADRIA Développement</b> ZA Creac'h Gwen 29196 Quimper Cedex (France)
<b>&gt; For:</b>	<b>bioMérieux</b> 376 Chemin de l'Orme 69280 Marcy l'Etoile (France)

This report consists of 91 pages, including 14 appendices.

Only copies including the totality of this report are authorised.

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

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Quality Assurance documents related to this study can be consulted upon request from **bioMérieux**.

Measurement uncertainty on the reference method results is not taken into account to provide the conclusion in this report; this measurement uncertainty is however available.

The technical protocol and the result interpretation were carried out according to EN ISO 16140-2:2016 and the AFNOR technical rules (PR Revision 7).

<b>Validation protocols</b>	<ul style="list-style-type: none"> <li>▪ ISO 16140-1 (2016): Microbiology of the food chain - Method validation — <i>Part 1: Vocabulary</i></li> <li>▪ ISO 16140-2 (2016): Microbiology of the food chain - Method validation — <i>Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method</i></li> <li>▪ AFNOR technical rules (PR Revision 7).</li> </ul>
<b>Reference method*</b>	NF EN ISO 16649-2 (July 2001): Horizontal method for the enumeration of $\beta$ glucuronidase positive <i>Escherichia coli</i> – Part 2: colony-count technique at 44°C using 5-bromo-4-chloro-3 indoly $\beta$ -D-glucuronate
<b>Alternative method</b>	<b>TEMPO® EC</b>
<b>Scope</b>	<input checked="" type="checkbox"/> All human food <input checked="" type="checkbox"/> Pet food
<b>Certification organization</b>	AFNOR Certification ( <a href="http://nf-validation.afnor.org/">http://nf-validation.afnor.org/</a> )

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\* Analyses performed according to the COFRAC accreditation

## 1 INTRODUCTION

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The TEMPO® EC method was validated in February 2005 for human food and pet food (Certificate number BIO 12/13-02/05) according to the ISO 16140 (2003) standard.

<b>February 2005</b>	Initial validation study for food and feed product
<b>May 2007</b>	Extension validation for a specific protocol for milk powders including the secondary diluents MOPS buffer instead of distilled water
<b>January 2009</b>	Renewal study
<b>February 2011</b>	Extension study to extend the scope of the validated TEMPO® EC method to raw milks. An accuracy study was run.
<b>November 2012</b>	Renewal study
<b>January 2017</b>	Renewal study according to the NF EN ISO 16140-2:2016
<b>December 2020</b>	Renewal study
<b>April 2023</b>	Extension study for a new protocol for dairy products with incubation of the cards at 44°C

## 2 METHOD PROTOCOLS

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### 2.1 Alternative method

#### 2.1.1 Principle

TEMPO® system is an automated method associating an innovative card with an adapted medium to ensure rapid enumeration of several quality indicators.

The method is based on the MPN principle (Most Probable Number), with the TEMPO® card.

The TEMPO® EC test consists in a culture medium associated to the TEMPO® card. The culture medium is inoculated with the sample to be tested and transferred by the TEMPO® Filler into the card.

Depending on the number and size of the positive wells, the TEMPO® Reader calculates the number of *E. coli*. Card reading, interpretation and reporting are directly managed by the TEMPO® system.

### 2.1.2 Protocol

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

For all categories (food and pet food):

- Incubation of the TEMPO cards for 24h-27 h at 37°C ± 1°C

For dairy products:

- Incubation of the TEMPO cards for 24h-27 h at 44°C ± 1°C
- Possibility to store the TEMPO cards for 48h at 5°C ± 3°C

### 2.1.3 Restriction

Using the TEMPO® EC method is not recommended to analyse the products with a high enzymatic activity. This restriction concerns particularly the raw mollusc and red offals, which needs a dilution rate >1/40.

## 2.2 Reference method♦

The standard method is the ISO 16649-2 (July 2001): Horizontal method for the enumeration of β glucuronidase positive *Escherichia coli* – Part 2: colony-count technique at 44°C using 5-bromo-4-chloro-3 indoly β-D-glucuronate.

The protocol of the reference method is given in **Appendix 2**.

## 2.3 Protocols applied during the validation studies

For the initial validation study (2005) and extensions performed in 2007 and 2011, the TEMPO cards were incubated for 24h at 37°C ± 1°C. For the extension study which concerns the dairy products category (2023), the TEMPO cards were incubated for 24 h at 44°C and read again after storage for 48h at 5°C ± 3°C.

## 3 INITIAL VALIDATION STUDY, EXTENSION/RENEWAL STUDIES: RESULTS

### 3.1 Method comparison study

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

*The study was carried out on a diversity of samples and strains representative of agri-food products. This does not constitute an exhaustive list of the different matrices included in the scope.*

*For any comment on the alternative method, please contact AFNOR Certification at <http://nf-validation.afnor.org/contact-2/>.*

#### 3.1.1 Relative trueness

*The relative trueness is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples.*

##### 3.1.1.1 Number and nature of the samples

The following samples were analysed:

Year	Number of samples analysed	Study
2004	73	Initial validation study
2006	30	Extension study for a specific protocol dedicated to milk powders
2010	19	Extension study for raw milks
2011	52	Data for bioMérieux (complementary data: meat products and composite foods)
2016	16	Renewal study

In 2004, 2006 and 2010, the analyses were realised in duplicate according to the ISO 16140 (2003).

A total of 190 samples were analysed providing 141 interpretable results. The number of samples tested per category and type is presented in table 1.

**Table 1 - Number and nature of the samples**

Category		Type	Number of samples analysed	Number of samples with interpretable results	
1	RTE & RTRH products	a	Ready to eat	10	9
		b	Ready to reheat	9	7
		c	Cooked delicatessen	5	5
		Total		24	21
2	Meat products	a	Raw meat, except poultry meat	7	7
		b	Raw poultry meat	11	11
		c	Delicatessen (to cook)	11	10
		Total		29	28
3	Dairy products	a	Cheeses and ice creams	8	6
		b	Milk powders	30	23
		c	Raw milk	19	10
		Total		57	39
4	Seafood products	a	Raw	13	5
		b	Ready to reheat	5	5
		c	Smoked	5	5
		Total		23	15
5	Fruits and vegetables	a	Raw, non-processed	12	5
		b	Ready to eat	16	10
		c	Ready to reheat	12	8
		Total		40	23
6	Pet food	a	Raw	5	5
		b	Sausages, pâtés	6	5
		c	Pellets	6	5
		Total		17	15
<b>ALL CATEGORIES</b>			<b>190</b>	<b>141</b>	

### 3.1.1.2 Artificial and natural contamination of the samples

Naturally contaminated samples were analysed preferably. Artificially contaminated samples, using spiking or seeding protocols, were also analysed. The inoculated strains and the contamination protocols are provided in **Appendix 3**.

Among the interpretable results, 64 samples were naturally contaminated, and 77 samples were artificially contaminated.

Note that all the artificially contaminated samples are indicated in bold typing all among the report.



### 3.1.1.3 Raw data

The raw data are provided in **Appendix 4**.

The samples were analysed by the reference and the alternative methods in order to have 15 interpretable results per category, and 5 interpretable results per tested type.

The data are classified in three categories (See Table 2):

- Interpretable results with the reference and the alternative methods.
- Results with less than 4 colonies per plate with the reference and/or the alternative method (indicated with “\*” in the data) in order to have a more precise result. These results are not included in the calculation.
- Results below or above the quantification limit: according to the ISO 16140-2:2016, if any result (either reference or alternative method) is below the quantification limit, the data should be plotted using a substituted value of  $1 \log_{10}$  units less than the observed value in case of a lower than value. Similarly, any value greater than the upper limit should be amended by adding 1 log unit. These results are not included in the calculations but also appear on the graphs.

**Table 2 - Classification of the data**

Category		Type	Number of samples analysed	Number of samples with < 4 CFU/plate	Number of samples below or above the detection limit	Number of samples with no result	Number of samples with interpretable results	
1	RTE & RTRH products	a	Ready to eat	10	0	0	0	9
		b	Ready to reheat	9	0	1	0	7
		c	Cooked delicatessen	5	0	2	0	5
		Total		24	0	3	0	21
2	Meat products	a	Raw meat, except poultry meat	7	0	0	0	7
		b	Raw poultry meat	11	0	0	0	11
		c	Delicatessen (to cook)	11	0	1	0	10
		Total		29	0	1	0	28
3	Dairy products	a	Cheeses and ice creams	8	1	1	0	6
		b	Milk powders	30	3	3	1	23
		c	Raw milk	19	2	7	0	10
		Total		57	6	11	1	39
4	Seafood products	a	Raw	13	0	7	1	5
		b	Ready to reheat	5	0	0	0	5
		c	Smoked	5	0	0	0	5
		Total		23	0	7	1	15
5	Fruits and vegetables	a	Raw, non-processed	12	0	7	0	5
		b	Ready to eat	16	1	5	0	10
		c	Ready to reheat	12	0	4	0	8
		Total		40	1	16	0	23
6	Pet food	a	Raw	5	0	1	0	5
		b	Sausages, pâtés	6	0	1	0	5
		c	Pellets	6	0	0	0	5
		Total		17	0	2	0	15
<b>ALL CATEGORIES</b>			<b>190</b>	<b>7</b>	<b>40</b>	<b>2</b>	<b>141</b>	

The samples, which were not used in the calculations, are provided in Table 3.

Table 3 - Samples, which were not used in the calculations

Sample N°	Product	Reference method: ISO 16649-2 <sup>♦</sup>	Alternative method: TEMPO EC	Category	Type
2122	<b>Smoked salmon</b>	<1.00	<1.00	1	a
160	Ready to reheat food	<1.00	<1.00	1	b
2061	<b>Ready to eat Chinese food</b>	<1.00	<1.00	1	b
1865	Frozen sausage	1.00*	<1.00	2	c
193	Raw cow milk cheese	1.39*	1.88	3	a
241	<b>Ice cream (red fruits)</b>	<1.00	2.11	3	a
2509	<b>Infant formula milk powder</b>	<1.00	<1.00	3	b
2510	<b>Infant formula milk powder</b>	0.85*	<1.00	3	b
2511	<b>Infant formula milk powder</b>	2.39	<1.00	3	b
2512	<b>Infant formula milk powder</b>	3.45	No result	3	b
2504	<b>Infant formula milk powder</b>	1.05*	1.00	3	b
2546	<b>Infant formula milk powder</b>	1.39*	1.00	3	b
2547	<b>Infant formula milk powder</b>	1.53*	1.16	3	b
4717	Raw cow milk	0.70*	0.70*	3	c
5055	Raw cow milk	0.70*	0.70*	3	c
4716	Raw cow milk	<1.00	<1.00	3	c
4913	Raw cow milk	<2.00	1.16	3	c
4914	Raw cow milk	<2.00	1.00	3	c
5056	Raw cow milk	<1.00	<1.00	3	c
5112	Raw goat milk	<1.00	<1.00	3	c
5113	Raw goat milk	<1.00	<1.00	3	c
5114	Raw goat milk	<1.00	<1.00	3	c
200	Raw Clams	<1.00	No result	4	a
201	Shells	<1.00	<2.00	4	a
221	Raw Oysters	<1.00	<2.00	4	a
222	Sardines	<1.00	<1.00	4	a
223	Trout	<1.00	<1.00	4	a
256	<b>Raw sea almonds</b>	<1.00	<2.00	4	a
257	<b>Raw clams</b>	<1.00	<2.00	4	a
258	<b>Raw oysters</b>	<1.00	<2.00	4	a
282	<b>Cabbage</b>	0.85*	<1.00	5	a
249	Buckwheat seed	<1.00	<1.00	5	a
259	<b>Carrots</b>	<1.00	<1.00	5	a
261	<b>Green cabbage</b>	<1.00	<1.00	5	a
262	Figs	<1.00	<1.00	5	a
269	Figs	<1.00	<1.00	5	a
282	<b>Cabbage</b>	0.85*	<1.00	5	a
283	<b>Mixed salad</b>	1.24*	1.00	5	b
263	Dried pears	<1.00	<1.00	5	b
267	Dried apricots	<1.00	<1.00	5	b
268	Pistachios	<1.00	<1.00	5	b
281	<b>Grated carrots</b>	<1.00	<1.00	5	b
293	<b>Dried apple</b>	<1.00	2.55	5	b
196	Ready to reheat vegetables	<1.00	<1.00	5	c
197	Ready to reheat vegetables	<1.00	<1.00	5	c
220	Ready to reheat vegetables	<1.00	<1.00	5	c
270	Dried pears	<1.00	<1.00	5	c
7205	<b>Sausage for dog</b>	2.00	<1.00	6	b
7206	<b>Pellets for cats</b>	1.96	<1.00	6	c

\* : &lt; 4 colonies/plate

♦ Analyses performed according to the COFRAC accreditation

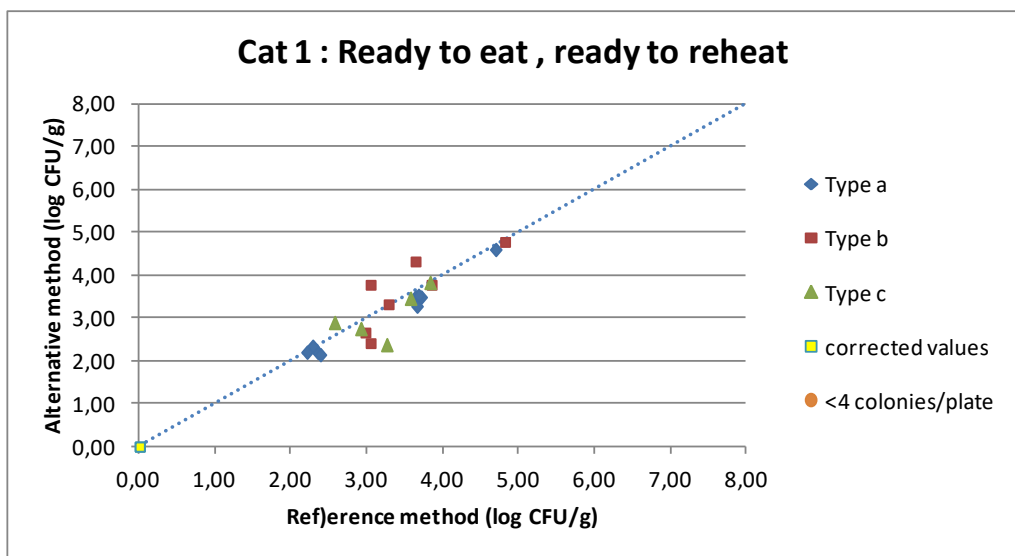
### 3.1.1.4 Statistical interpretation

The calculations are provided in **Appendix 5**.

The obtained data were analysed using the scatter plot. The graphs are provided with the line of identity ( $y = x$ ):

The Figures 3 to 8 show the data plotted for each individual category. The Figure 9 shows the data plotted for all the products.

**Figure 1 - Data plotted for Ready to eat and ready to reheat products**



**Figure 2- Data plotted for Meat products**

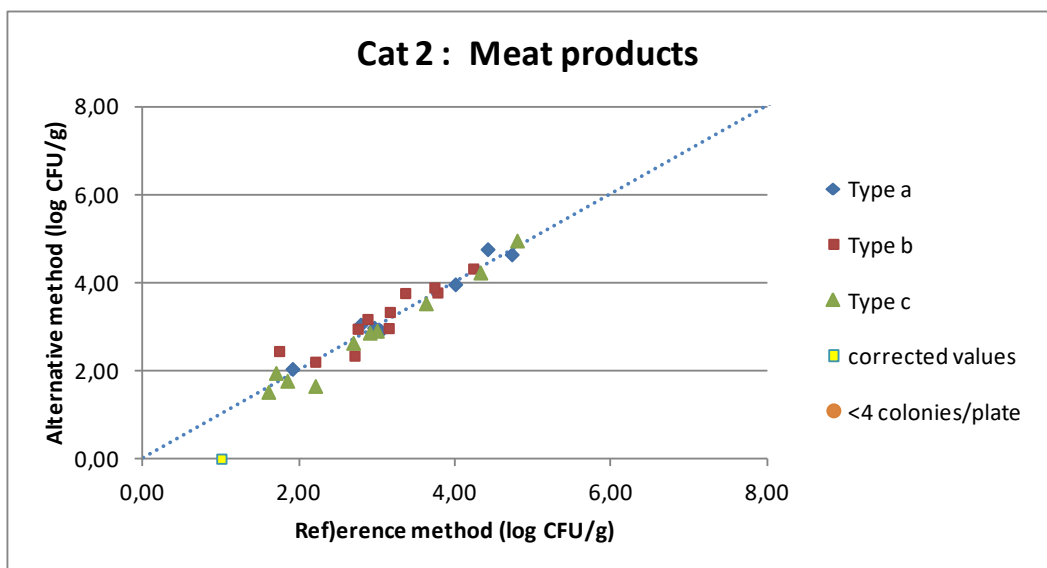


Figure 3- Data plotted for Dairy products

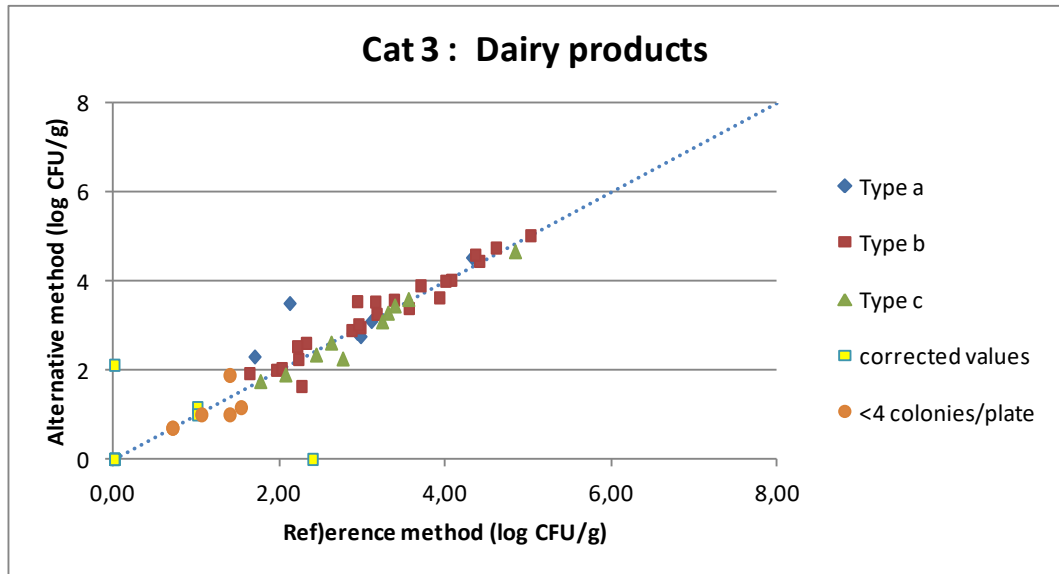


Figure 4- Data plotted for Seafood products

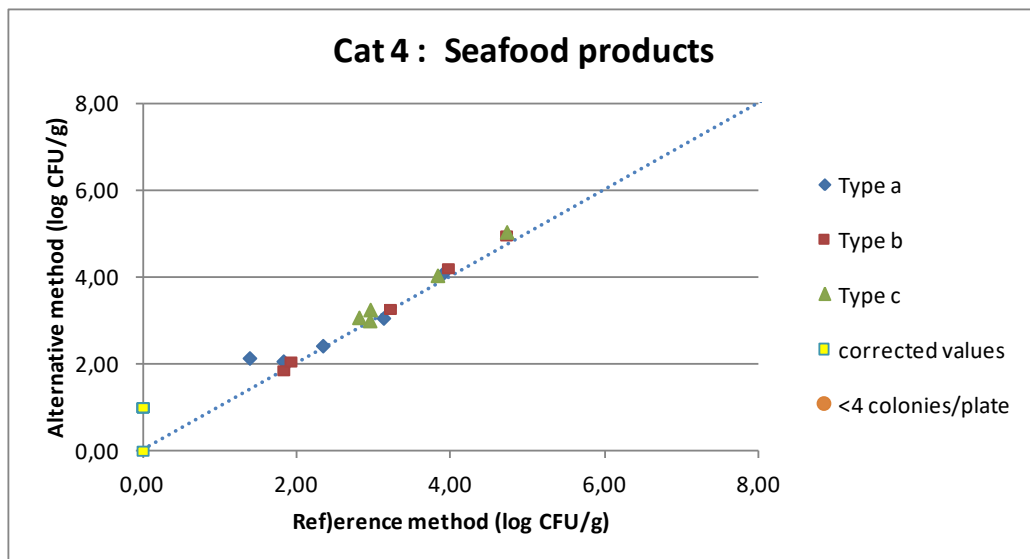


Figure 5- Data plotted for **Fruits and vegetables**

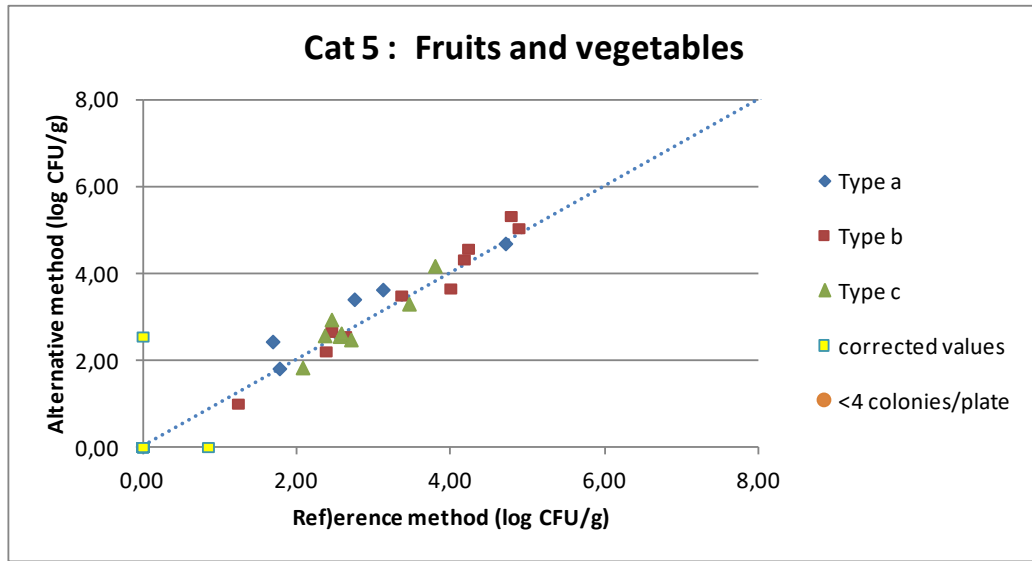
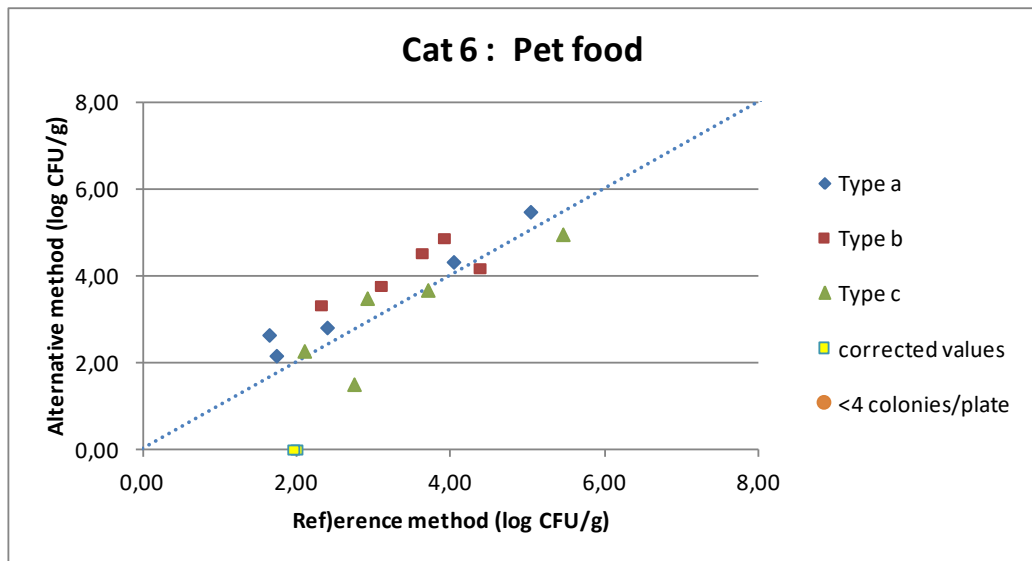
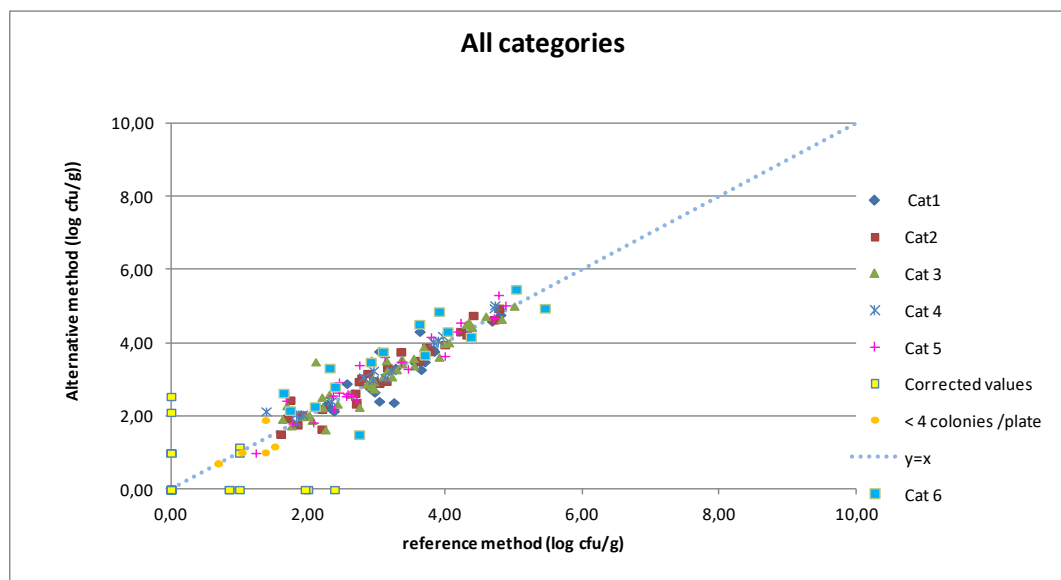


Figure 6- Data plotted for **Pet food**



**Figure 7 - Data plotted for all the products**



The calculated values for Average difference and Standard deviation differences per category are provided in Table 4.

**Table 4 - Calculated values per category**

Category		n	$\bar{D}$	SD	95% lower limit	95% upper limit
1	RTE & RTRH	21	-0,08	0,36	-0,85	0,69
2	Meat products	28	0,05	0,25	-0,46	0,56
3	Dairy products	39	0,07	0,32	-0,59	0,74
4	Seafood products	15	0,20	0,19	-0,22	0,62
5	Fruits and vegetables	23	0,13	0,31	-0,52	0,79
6	Pet food	15	0,32	0,62	-1,06	1,70
<b>All categories</b>		<b>141</b>	<b>0,09</b>	<b>0,36</b>	<b>-0,62</b>	<b>0,81</b>

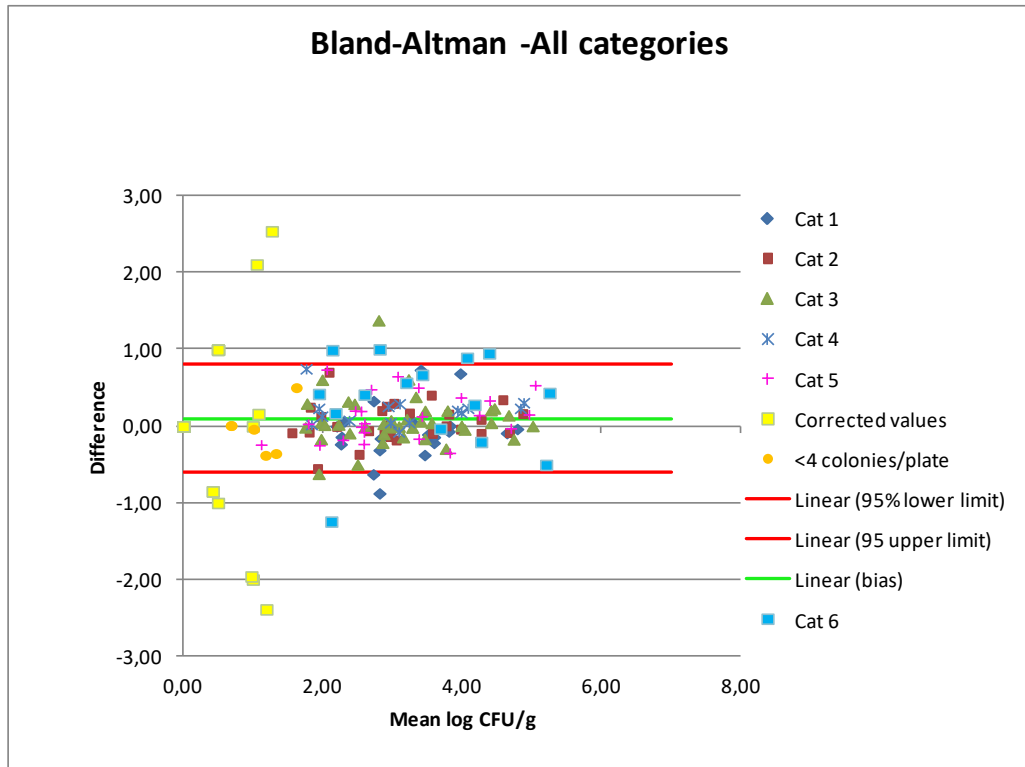
$\bar{D}$ : Average difference

SD: Standard deviation of differences

The bias varies from -0,08 log CFU/g to 0,32 log CFU/g. For all combined categories, the bias is 0,09 log CFU/g.

The Bland-Altman difference plot for all the samples is given Figure 10.

Figure 8 – Bland-Altman difference plot for all the samples



Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits are listed in the Table 5.



**Table 5 - Analysis of the data out of the confidence limits**

Values in **green**: differences in favour of the alternative method

Values in **red**: differences in favour of the reference method

Values in **black**: equivalent enumeration observed with both methods

	Corrected value
	Results calculated using enumeration lower than 4 CFU/plate

Classification of data	Category	Type	N° Sample	Product	Reference method		Alternative method		Mean	Difference
					Corrected values	True values	Corrected values	True values		
Interpretable data	1	c	2369	Cooked ham	/	3.26	/	2.38	2.82	-0.88
	6	c	7207	Pellets for cats	/	2.75	/	1.51	2.13	-1.24
	3	a	194	Raw goat milk cheese	/	2.11	/	3.50	2.80	1.38
	6	a	226	Ground beef for animals	/	1.65	/	2.64	2.14	0.99
	6	b	272	Sausage for dog	/	2.32	/	3.32	2.82	1.00
	6	b	273	Pâté for dog	/	3.63	/	4.52	4.08	0.89
	6	b	274	Pâté for dog	/	3.92	/	4.87	4.39	0.95
< or > quantification limit	2	c	1865	Frozen sausages	/	1.00	0.00	<1.00	0.50	-1.00
	3	b	2510	Infant formula	/	0.85	0.00	<1.00	0.42	-0.85
	3	b	2511	Infant formula	/	2.39	0.00	<1.00	1.19	-2.39
	5	a	282	Cabbage	/	0.85	0.00	<1.00	0.42	-0.85
	6	b	7205	Sausage for dog	/	2.00	0.00	<1.00	1.00	-2.00
	6	c	7206	Pellets for cats	/	1.96	0.00	<1.00	0.98	-1.96
	3	a	241	Ice cream (red fruits)	0.00	<1.00	/	2.11	1.06	2.11
	4	a	201	Raw shells	0.00	<1.01	1.00	<2.00	0.50	1.00
	4	a	221	Raw oysters	0.00	<1.00	1.00	<2.00	0.50	1.00
	4	a	223	Trout	0.00	<1.00	1.00	<2.00	0.50	1.00
	4	a	256	Raw sea almonds	0.00	<1.00	1.00	<2.00	0.50	1.00
	4	a	257	Clams	0.00	<1.00	1.00	<2.00	0.50	1.00
	5	b	293	Dried apples	0.00	<1.00	/	2.55	1.27	2.55

### 3.1.1.5 Discordant results

20 samples are outside of the 95 % confidence limits. Their repartition is given in Table 6.

**Table 6 - Repartition of the samples outside of the 95 % Confidence Limits**

		Number of samples
Interpretable results by both methods	< LCL	2
	> UCL	5
	Total	7
< or > the quantification limit	< LCL	6
	> UCL	7
	Total	13
<b>Total &lt; LCL</b>		<b>8</b>
<b>Total &gt;UCL</b>		<b>12</b>
<b>Total</b>		<b>20</b>

For a majority of samples (13), the differences observed between the reference and the alternative methods are explained by the fact that one of the two results is below the detection limit.

In the other cases (7 samples concerned), the difference was in favour of the alternative method (5 times) and in favour of the reference method (2 times). The differences varied from - 1.24 to 1.38 log.

### 3.1.1.6 Conclusion

**The relative trueness of the alternative method is satisfying.**

### 3.1.2 Accuracy profile

The accuracy profile is a graphical representation of the capacity of measurement of the quantitative method, obtained by combining acceptability intervals and  $\beta$ -expectation tolerance intervals, both reported to different levels of the reference value.

#### 3.1.2.1 Experimental design

Six matrix/strain pairs were tested. A minimum of one type per category, and therefore 2 different batches, were selected, using 6 samples per type. 2 samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. In the end, 30 samples were tested per matrix type.

The following matrix/strain pairs were studied (See Table 7).

**Table 7 - Matrix/strain pairs**

	Category	Matrix	Inoculated strain	Origin	Inoculation level CFU/g
1	Ready to eat and ready to reheat	Pâté	<i>E. coli</i> 144	Ready to reheat meal	100
2	Meat products	Ground beef	<i>E. coli</i> 13	Ground beef	5 000
3	Dairy products	Pasteurized milk	<i>E. coli</i> 94	Cheese	
4	Fruits and vegetables	Grated carrots	<i>E. coli</i> 19	Grated carrots	100 000
5	Seafood	Raw fish filet	<i>E. coli</i> Ad228	Fish	
6	Pet food	Dog pâté	<i>E. coli</i> 123	Veal liver	

#### 3.1.2.2 Calculation and interpretation

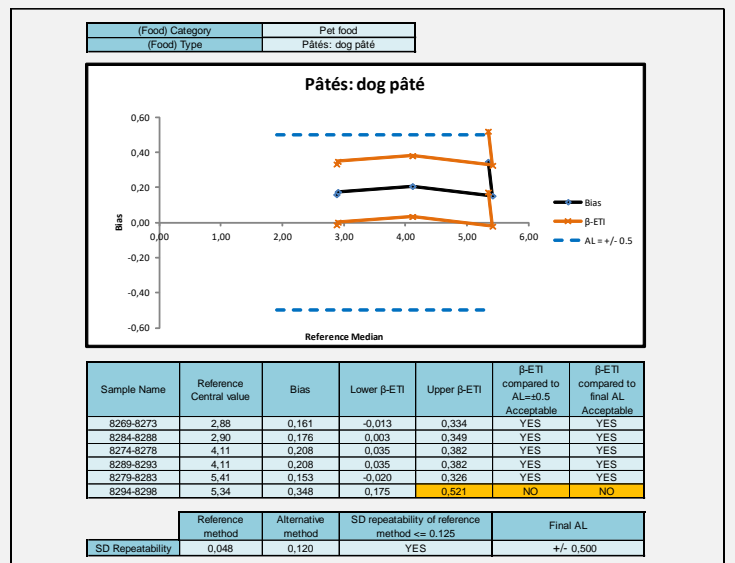
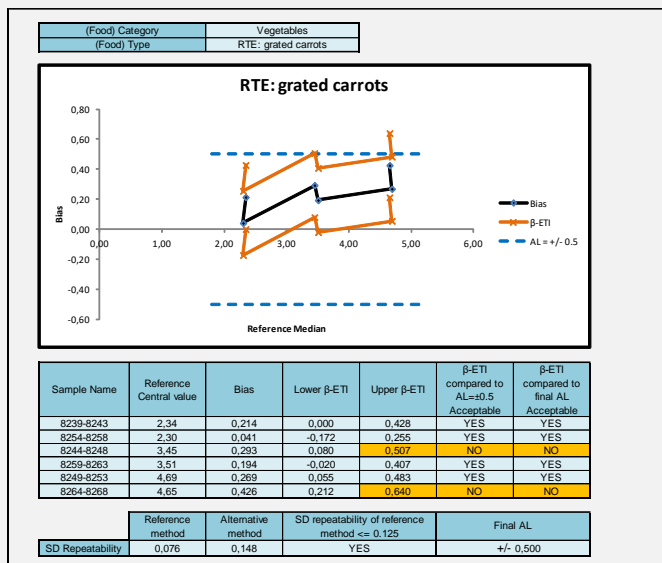
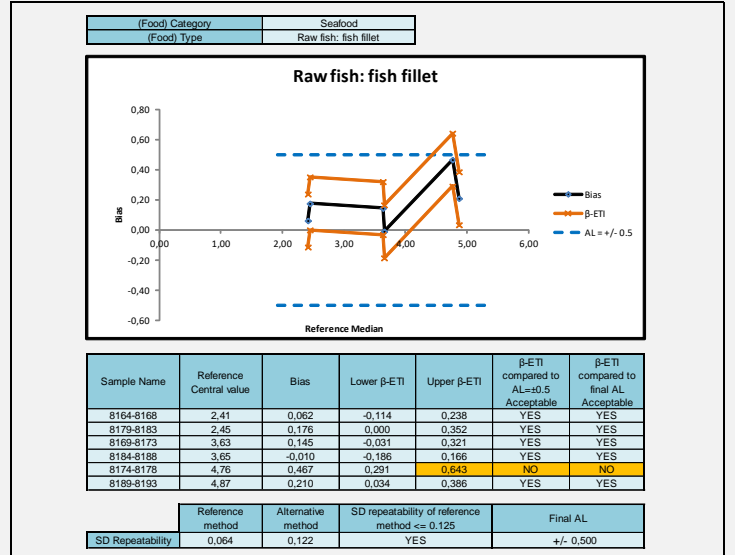
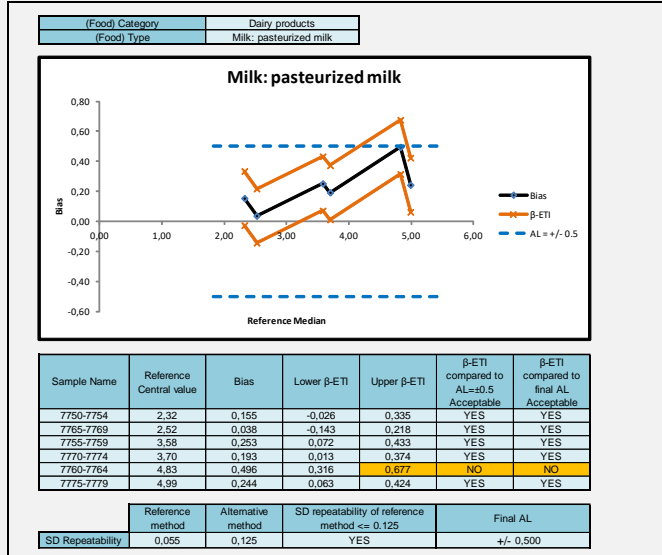
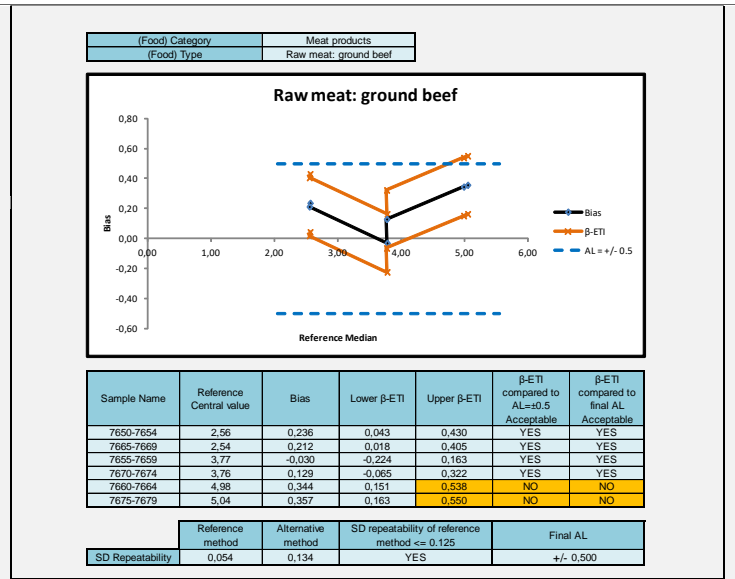
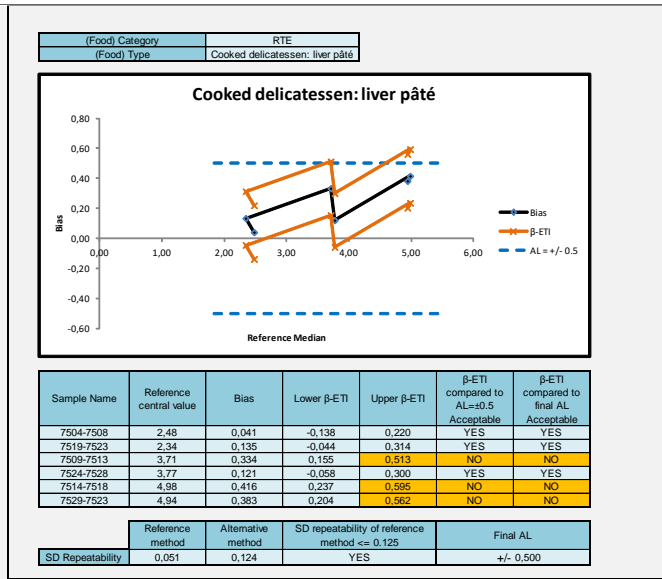
The raw data are provided in **Appendix 6** and the summary tables (in log CFU/g) in **Appendix 7**. The statistical results and the accuracy profiles are provided in Figure 11.

The calculations were done using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) ver 2027-01-2015 available on <http://standards.iso.org/iso/16140>

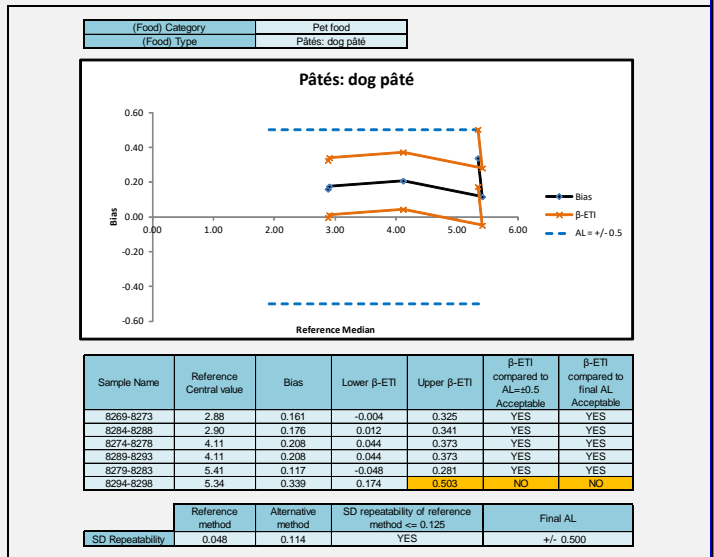
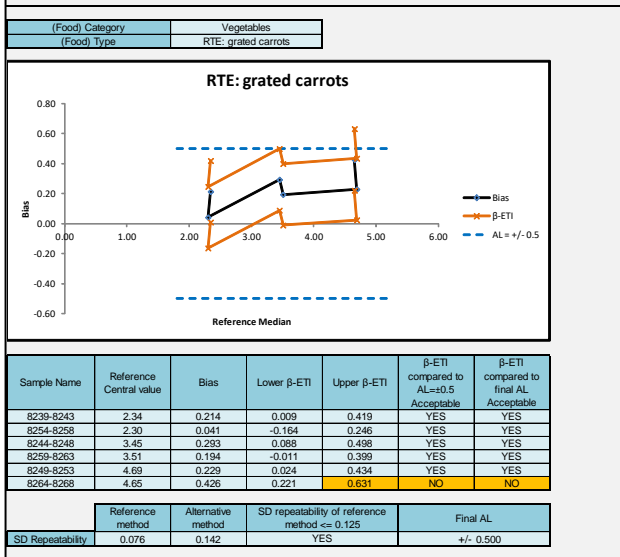
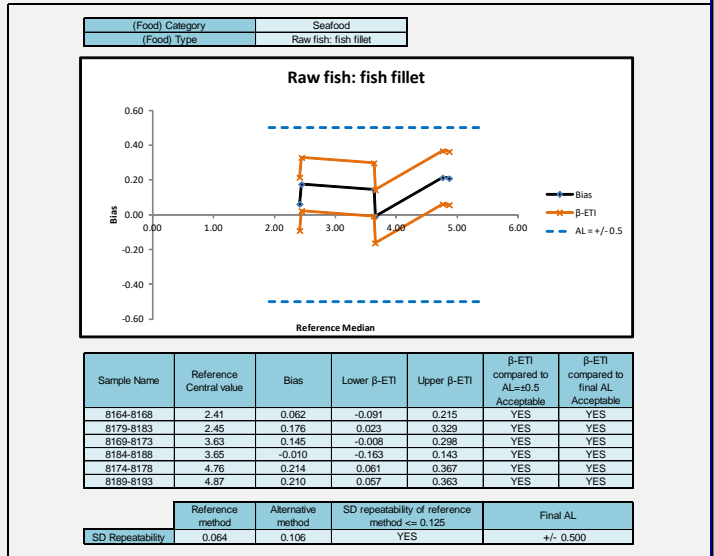
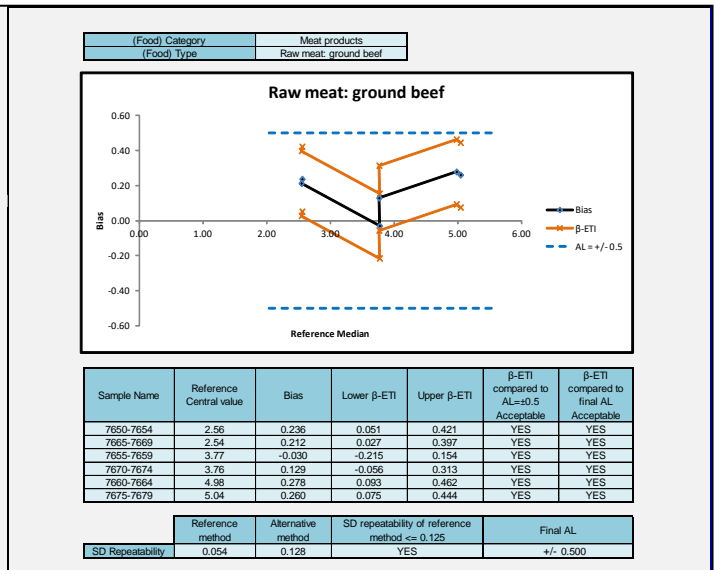
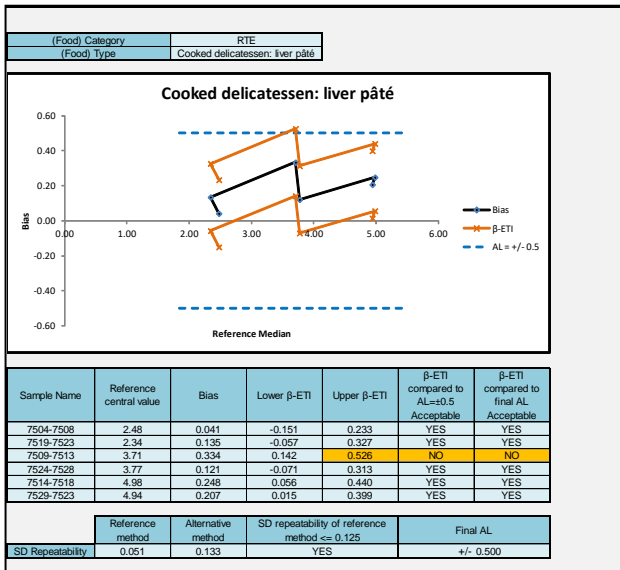
The following interpretations were done:

- Level 1: 1/40                      Level 2: 1/400 when available                      Level 3: 1/400
- Level 1: 1/40                      Level 2: 1/4000    Level 3: 1/4000

Figure 9 – Accuracy profile  
Dilution 1/40 / 1/400



# Accuracy profile Dilution 1/40 / 1/4000



When the 1/40 and 1/400 dilutions are used for interpretation, the upper limit is above the acceptability limit for the high inoculation level for all the tested matrices. For four matrices this concerns only one matrix batch among the two tested. For the lower and the medium inoculation levels, the limits are within the acceptability limits for the six matrix strain pairs tested.

Contamination levels observed on naturally contaminated samples are generally very low. The criteria for *E. coli* are comprised between 1 CFU to < 500 CFU/g (FCD, Commission Regulation N°2013/2015 on microbial criteria for foodstuffs). According the AFNOR Technical Rules (Version 5) inoculation levels between 300 and 10<sup>5</sup> CFU were tested. This resulted for using the 1/400 or 1/4000 dilutions.

When 1/40 and 1/4000 dilutions are used, the upper limit is above the acceptability limit for:

- Liver pâté, Level 2 (Upper  $\beta$  ETI = 0.526);
- Pasteurized milk, Level 3 (Upper  $\beta$  ETI = 0.532 and 0.657).

A positive bias due to a better recovery of *E. coli* with TEMPO EC method compared to ISO 16649-2 was previously observed and demonstrated.

If the average bias is subtracted from the values obtained for  $\beta$  ETI for these two matrices, the upper limits ( $D = 0.1$  log) are within the acceptability limits for all the tested matrices.

For the renewal study run in 2012, additional testing run on a whipped cream from pastry was tested. Using the two reference methods (ISO 16140 Part 1 (membrane enumeration) and Part 2 (pour plate enumeration) and the TEMPO<sup>®</sup> EC method, the following results were obtained:

Product	ISO 16649-1	ISO 16649-2	TEMPO EC
Whipped cream from "Baba au rhum"	3.69	2.92	3.38

The ISO 16649-1 is recommended for analysis of samples supposed to contain injured bacteria. The enumeration observed with TEMPO<sup>®</sup> EC is close to the enumeration obtained with the ISO 16649-1 indicating that the TEMPO<sup>®</sup> EC method has better performances than the ISO 16649-2 for enumeration of injured bacteria. This could explain the bias observed for the accuracy profiles.

An article from Baylis, Green, Betts (2006) confirms this hypothesis: 57 samples (fresh or frozen) were tested using the ISO 16649-1, ISO 16649-2 and the TEMPO<sup>®</sup> EC method. The ISO 16649-1 showed a better recovery of the injured cells, the enumeration obtained with the TEMPO<sup>®</sup> method were similar to those of the ISO

16649-1 standard and the lowest enumerations were obtained using the ISO 16649-2 standard.

### 3.1.2.3 Conclusion

**Considering the positive bias of TEMPO EC method, the accuracy profiles are comprised within the acceptability limits when the 1/40 and 1/4000 dilutions are used, in agreement with the level of contamination of samples.**

### 3.1.3 Inclusivity and exclusivity

*The inclusivity study involves pure target strains to be detected or enumerated by the alternative method. The exclusivity study involves pure non-target strains, which can be potentially cross-reactive, but are not expected to be detected or enumerated by the alternative method.*

#### 3.1.3.1 Protocols

During the initial validation, 30 target strains and 20 non target strains were tested *in duplicata* with the compared methods. The pure cultures were as well enumerated on non-selective agar, *i.e.* PCA.

20 additional target strains and 10 non-target strains were tested for the renewal study.

#### 3.1.3.2 Results

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

The *E. coli* strains which shown characteristic colonies on TBX ( $\beta$ -glucuronidase positive) were detected by the TEMPO<sup>®</sup> EC method.

For most of the tested target strains, the enumeration results are a little bit higher with the TEMPO<sup>®</sup> EC method in comparison to the ISO 16649-2.

No cross reaction was observed with the non-target strains for both methods, *i.e.* TEMPO<sup>®</sup> EC and ISO 16649-2.

**TEMPO EC method shows satisfying specificity and selectivity.**

### 3.1.4 Limit of quantification (LOQ)

*The limit of Quantification (LOQ) is the lowest analyte concentration that can be quantified with an acceptable level of precision and trueness under the conditions of the test.*

The LOQ was determined as is it needed for the instrumental methods which are related to the growth of the microorganism.

#### 3.1.4.1 Experimental design

Blank samples were tested for each category. These blank samples were used to verify the limit of quantification of the alternative method. 10 test portions from the same sample were analysed. The same food type matrices than those tested in the accuracy profile were used.

#### 3.1.4.2 Calculation and interpretation

The threshold standard deviation  $S_0$  was calculated as followed:

$$s_0 = \frac{1}{n-1} \sum_{j=1}^n (y_j - \bar{y})^2$$

where:

$n$  = the total number of test portions used

$y_j$  = the log transformed result of test portion  $j$

$y$  = the average log transformed result of all test portions

The limit of quantification is calculated as  $LOQ = 10 s_0$ .

#### 3.1.4.3 Results

Raw data and calculation are provided in **Appendix 9**. The results are summarized in Table 8.



**Table 8 - Quantification limits per tested matrix**

Matrix	S <sub>0</sub>	LOQ
Liver pâté	0	0
Pâté for dog	0	0
Grated carrots	0	0
Pasteurized milk	0	0
Fish fillet	0	0
Ground beef	0	0

### 3.1.5 Practicability

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

<b>Storage conditions, shelf-life and modalities of utilisation after first use</b>	The storage temperatures are between 2 and 25°C (room temperature before analysis) depending on the materials and the reagents.		
<b>Time to result</b>	Steps	<b>Reference method</b>	<b>Alternative method</b>
	<b>Negative or positive samples</b>		
	Sampling	Day 0	Day 0
	Reading	Day 1	Day1
<b>Common step with the reference method</b>	Initial suspension		

The results are available in one day by both reference and alternative methods.

### 3.1.6 Conclusion

**For the initial and extension studies concerning all food products and pet food categories with incubation of the TEMPO cards at 37°C:**

- **190 samples were tested in the relative trueness study providing 141 interpretable results by both the reference and the TEMPO EC methods, which clearly satisfied the required criteria for quantitative method comparison per ISO 16140-2.**
- **The observed profiles are comprised within the AL actually set at 0.5 Log CFU/g in the EN ISO 16140-2:2016 for the 6 matrix/strain pairs in the range of contamination usually observed for *Escherichia coli* enumerations.**
- **The inclusivity and exclusivity testing shows satisfying results.**

## 3.2 Extension study (2023) with a new protocol for dairy products with incubation of the cards at 44°C

The flow diagram of the alternative method used for the extension study performed in 2023 is given in **Appendix 1**.

### 3.2.1 Relative trueness study

*The relative trueness is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples.*

#### 3.2.1.1 Number and nature of the samples

Only the dairy products category was concerned by the extension study. The repartition per tested type is provided in **Table 9**.

**Table 9 – Number and nature of the samples**

Category	Type	Items	Number of analysed	Number of samples with interpretable results		
				Cards incubated 24h at 44°C ± 1°C	24 h at 44°C + 48 h at 5°C ± 3°C	
Dairy products	a	Raw milk dairy products	Raw milk cheeses, raw milk, ...	12	9	9
	b	Dehydrated milk products	Milk powders and infant powders with and without probiotics	15	7	7
	c	Other dairy products	Pasteurized cheese, ice cream, ...	6	6	6
	<b>Total</b>			<b>33</b>	<b>22</b>	<b>22</b>

33 samples were analysed, leading to 22 exploitable results.

#### 3.2.1.2 Artificial and natural contamination of the samples

Artificial contaminations were realized by spiking or seeding protocols. The inoculated strains, the contamination protocols, the injured protocols of the inoculated vegetative cells and the injury evaluation are provided in **Appendix 10**. Injury efficiency was evaluated by enumerating the pure culture on selective and non-selective agars.

33 samples were analysed; 21 were artificially contaminated by seeding protocol, 9 by spiking protocol and 3 were naturally contaminated.

Among the 22 interpretable results, all were artificially contaminated.

### 3.2.1.3 Raw data

The raw data are provided in **Appendix 11**.

The samples were analyzed by the reference and the alternative methods in order to have 15 interpretable results, and 5 interpretable results per tested type.

The data are classified in three categories (See **Table 10**):

- Interpretable results with the reference and the alternative methods.
- Results with less than 4 colonies per plate with the reference method (indicated with “\*” in the data) in order to have a more precise result. These results are not included in the calculation.
- Results below or above the quantification limit: according to the ISO 16140-2:2016, if any result (either reference or alternative method) is below the quantification limit, the data should be plotted using a substituted value of 1 log<sub>10</sub> units less than the observed value in case of a lower than value. Similarly, any value greater than the upper limit should be amended by adding 1 log unit. These results are not included in the calculations but also appear on the graphs.

**Table 10 - Classification of the data**

Category	Type	Number of tested samples	Number of samples with interpretable results by both methods	Number of samples with less than 4 colonies/plate	Number of samples below or above the quantification limit	
Dairy products	a	Raw milk dairy products	12	9	0	3
	b	Dehydrated milk products	15	7	1	7
	c	Other dairy products	6	6	0	0
	<b>Total</b>		<b>33</b>	<b>22</b>	<b>1</b>	<b>10</b>

The samples, which were not used in the calculations for incubation 24h at 44°C and after storage of the cards for 48h at 5°C ± 3°C, are provided in **Table 11**.

**Table 11 - Samples which were not used in the calculations**

Sample N°	Product	Reference method (log CFU/g)	Alternative method (log CFU/g)	Category	Type
1007	Raw ewe milk	<1.00	0	1	a
1008	Raw goat milk	<1.00	0	1	a
1009	Raw ewe milk cheese	<1.00	<1.00	1	a
833	Milk powder	<1.00	<1.00	1	b
834	Milk powder	<1.00	<1.00	1	b
835	Infant formula without probiotics	<1.00	<1.00	1	b
836	Infant formula without probiotics	<2.00	1.00	1	b
838	Infant formula with probiotics	1.43*	1.00	1	b
1026	Infant formula with probiotics	<1.00	<1.00	1	b
1027	Infant formula without probiotics	<1.00	<1.00	1	b
1131	Infant formula with probiotics	2,62	<1.00	1	b

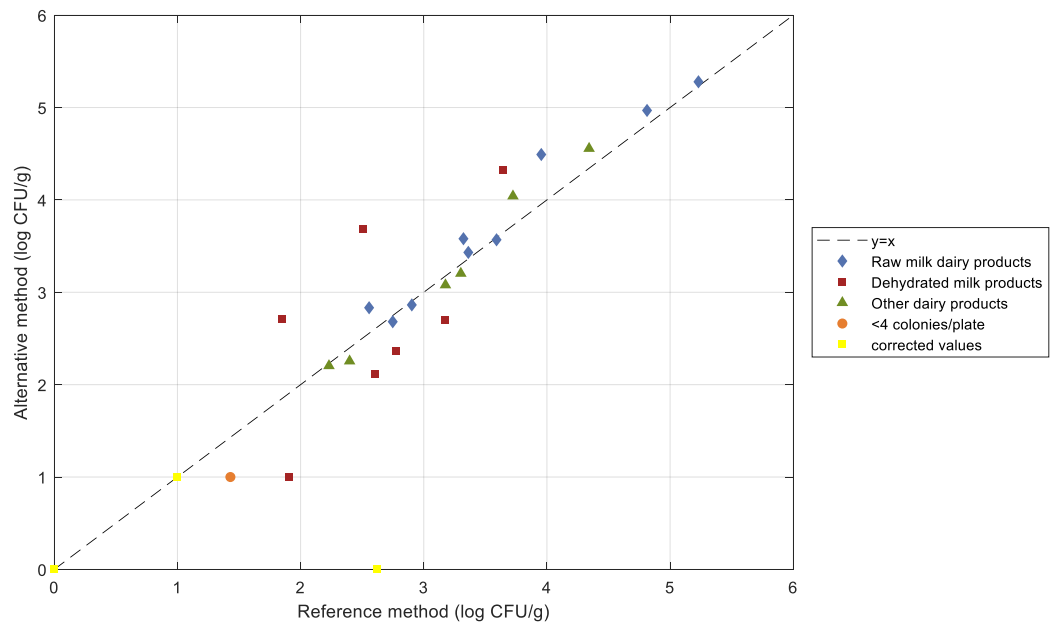
\*: Results with less than 4 colonies per plate with the reference method

#### 3.2.1.4 Statistical interpretation

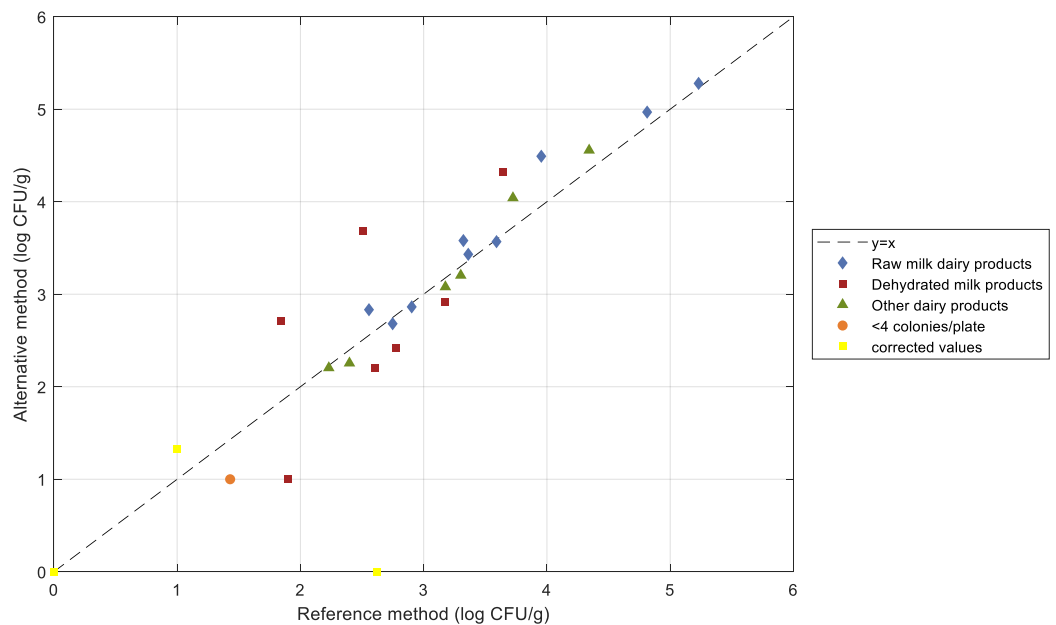
The obtained data were analyzed using the scatter plot. The graphs are provided with the line of identity ( $y = x$ ).

The **Figure 10** and **Figure 11** show the data plotted for the Dairy products category. The **Figure 12** shows the data plotted for all categories combined (*excluding dairy products*) tested at 37°C (24 h incubation time) with the dairy products category tested at 44°C.

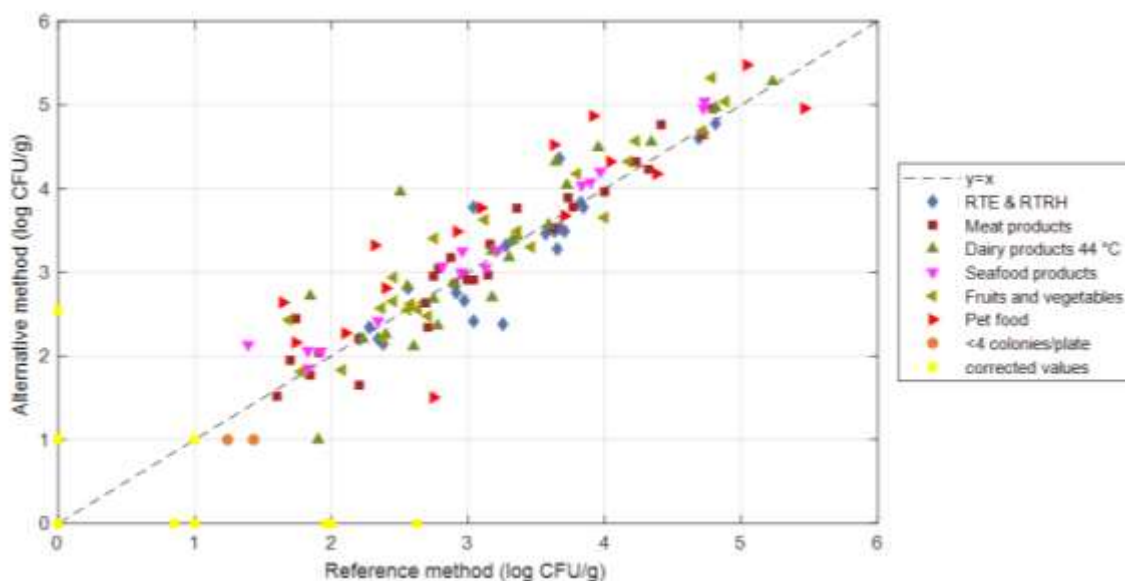
**Figure 10 - Data plotted for the Dairy products category  
24 h at 44°C ± 1°C**



**Figure 11 - Data plotted for the Dairy products category  
24 h at 44°C ± 1°C + 48h at 5°C ± 3°C**



**Figure 12 - Data plotted for all categories (24 h at 37°C± 1°C) and dairy products category (44°C 24 h at 44°C ± 1°C)**



The calculated values for Average difference and Standard deviation differences per category are provided in **Table 12**.

**Table 12 - Calculated values**

Category	Incubation time and temperature	n	$\bar{D}$ (Linear bias)	SD	95% lower limit	95% upper limit
Dairy products	24 h at 44°C ± 1°C	22	0.08	0.47	-0.90	1.07
	24 h at 44°C + 48 h at 5°C ± 3°C	22	0.10	0.45	-0.85	1.05
All categories	24 h at 37°C ± 1°C (all categories except dairy), 24 h at 44°C ± 1°C (dairy)	123	0.10	0.39	-0.68	0.89

$\bar{D}$ : Average difference (linear bias)      SD: Standard deviation of differences

The bias observed after incubation and after storage of the TEMPO cards, is closed to 0 for the dairy category tested at 44°C.

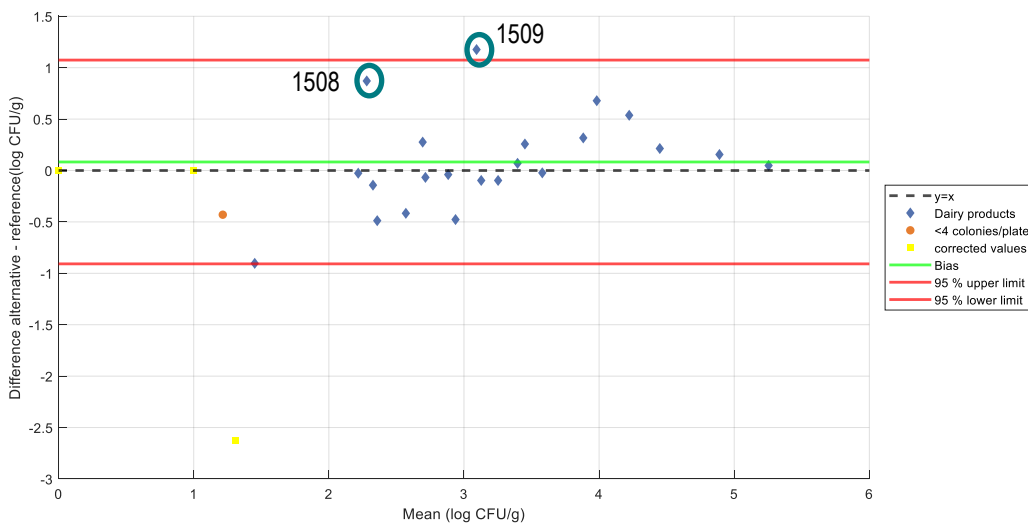
The bias for all categories when the data observed at 37°C for dairy products are replaced by the data obtained at 44°C is 0.10 log CFU/g.

Note that the bias observed for the dairy products category and for all categories combined when the cards were incubated at 37°C were respectively 0.07 and 0.09 log CFU/g.

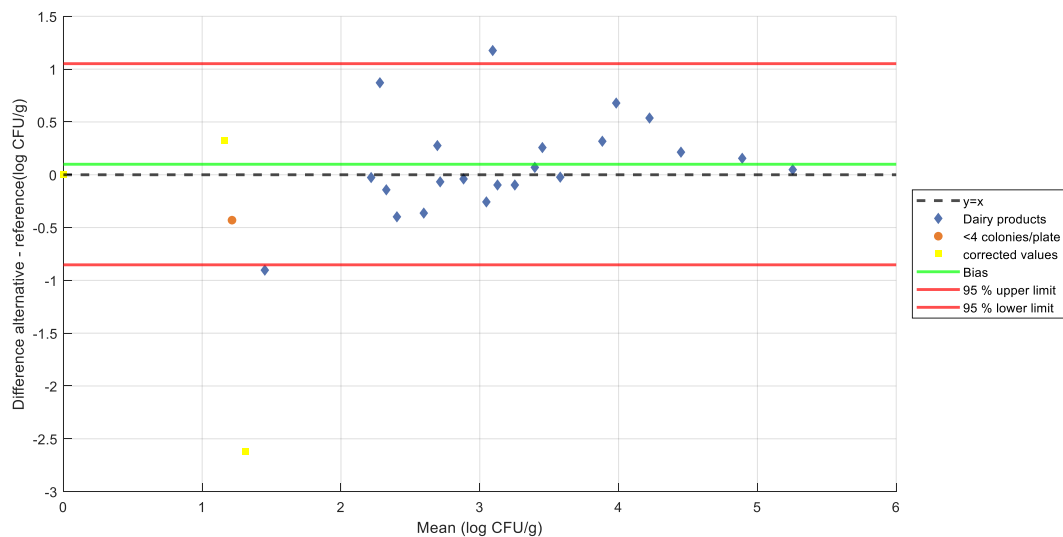
The Bland-Altman difference plot for the dairy category tested at 44°C are given **Figure 13** (after incubation time) and **Figure 14** (after cards storage).

The Bland-Altman difference plot for all categories with an incubation temperature at 37°C, except for dairy category tested at 44°C is given **Figure 15** (after incubation time).

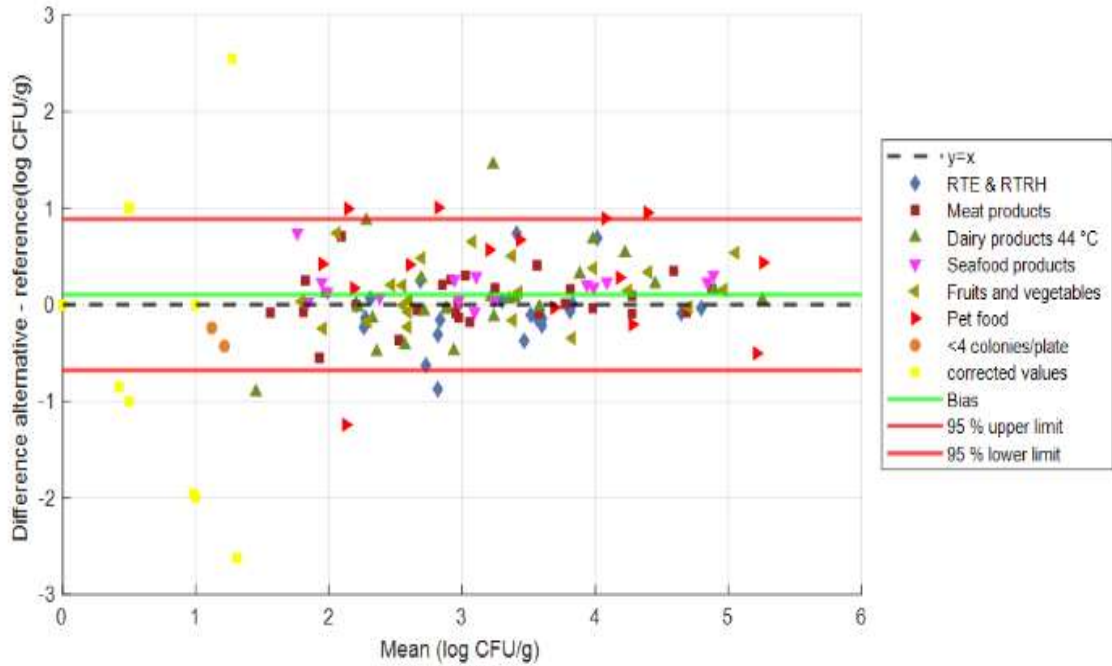
**Figure 13 – Bland-Altman difference plot for dairy products  
24 h at 44°C ± 1°C**



**Figure 14 – Bland-Altman difference plot for dairy products  
24 h at 44°C ± 1°C + 48h at 5°C ± 3°C**



**Figure 15 – Bland-Altman difference plot for all categories tested at 37°C ± 1°C except dairy products category tested at 44°C ± 1°C**





Samples for which the difference between the result observed with the reference and the alternative methods is above or lower than the limits for the dairy category tested at 44°C are listed in **Table 13**.



**Table 13 - Analysis of the data out of the confidence limits**

Values in **green**: differences in favour of the alternative method  
 Values in **red**: differences in favour of the reference method  
 Values in **black**: equivalent enumeration observed with both methods

 Corrected value  
 Results calculated using enumeration lower than 4 CFU/plate  
 UCL Upper confidence limit  
 LCL Lower confidence limit

Incubation time and temperature	Classification of data	Category	Type	Sample No	Product	Reference method	Alternative method	Values before correction (Reference or/and alternative method)	Mean	Difference	Lower / Upper Limits (CLs)
24 h at 44°C ± 1°C	Interpretable data	1	b	1509	Infant formula without probiotics	2,50	3,68	/	3,09	1,18	-0.91/1.07
	< or > quantification limit	1	b	1131	Infant formula with probiotics	2,62	0.00	1.00	1,31	-2,62	
24 h at 44°C ± 1°C + 48 h at 5° ± 3°C	Interpretable data	1	b	1509	Infant formula without probiotics	2.50	3.68	/	3.09	1.18	-0.85/1.05
		1	b	1025	Milk powder	1.90	1.00	/	1.45	-0.90	
	< or > quantification limit	1	b	1131	Infant formula with probiotics	2.62	0.00	1.00	1.31	-2.62	

### 3.2.1.5 Discordant results

The number of samples outside the LCs are classified in three categories (See **Table 14**).

**Table 14 - Classification of the samples outside of the CLs**

		Number of samples	
		24 h at 44°C ± 1°C	24 h at 44°C ± 1°C + 48 h at 5° ± 3°C
Interpretable results by both methods	< LCL	0	1
	> UCL	1	1
	Total	1	2
<4 CFU/plate	< LCL	0	0
	> UCL	0	0
	Total	0	0
< or > the quantification limit	< LCL	1	1
	> UCL	0	0
	Total	0	1
Total < LCL		1	2
Total >UCL		1	1
<b>TOTAL</b>		<b>2</b>	<b>3</b>

For samples 1508 (milk powder) and 1509 (infant formula with probiotics), the enumeration was clearly in favour of the TEMPO EC method, respectively 1.45 and 0.87 log difference after 24 h incubation. The enumeration observed for the TEMPO EC method fits better with the inoculation level of the *E. coli* strain in the matrix. The target inoculation levels as well as the enumeration of *E. coli* using the ISO 16649-2 and the TEMPO EC method for these two samples are given in **Table 15**.

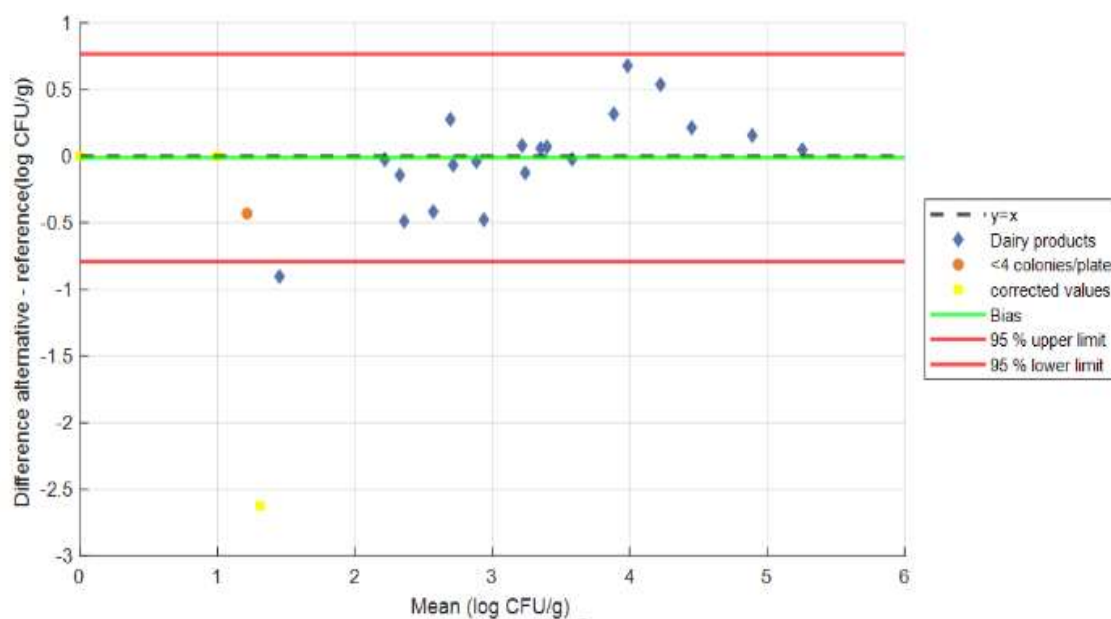
**Table 15 – Samples showing significant higher *E. coli* enumeration with the TEMPO EC method**

Sample N°	Product	Target inoculation level CFU/g	ISO 16649-2* CFU/g	TEMPO EC CFU/g
1508	Milk powder	1000	70	520
1509	Infant formula with probiotics	5000	520	9100

These discrepancy between the ISO 16649-2 and the TEMPO EC enumerations could be explained by the high selectivity of the TBX medium used in the ISO 16649-2 method.

The interpretation excluding these samples from the interpretation for the dairy category tested at 44°C is given **Figure 7**. In this case, the CLS were reduced to  $\pm 0.77$  log.

**Figure 16 – Bland-Altman difference plot for dairy products  
24 h at 44°C  $\pm$  1°C without samples 1508 and 1509**



### 3.2.1.6 Conclusion

**The relative trueness study of the alternative method is satisfying. The alternative method is reliable when compared to the reference method after incubation of the TEMPO cards for 24 h at 44°C  $\pm$  1°C and after storage of the cards for 48h at 5°C  $\pm$  3°C**

### 3.2.2 Accuracy profile study

The accuracy profile is a graphical representation of the capacity of measurement of the quantitative method, obtained by combining acceptability intervals and  $\beta$ -expectation tolerance intervals, both reported to different levels of the reference value.

#### 3.2.2.1 Matrices

One matrix was tested with three contamination levels and five test portions per level. The tested category, type, matrix and inoculated strain are provided in **Table 16**.

**Table 16 - Category, type and matrix**

Category	Type	Matrix	Strain	Origin	Target inoculation level (CFU/g)
Dairy products	c	Pasteurized cow milk cheese	<i>E. coli</i> 94	Cheese	100 5000 100000

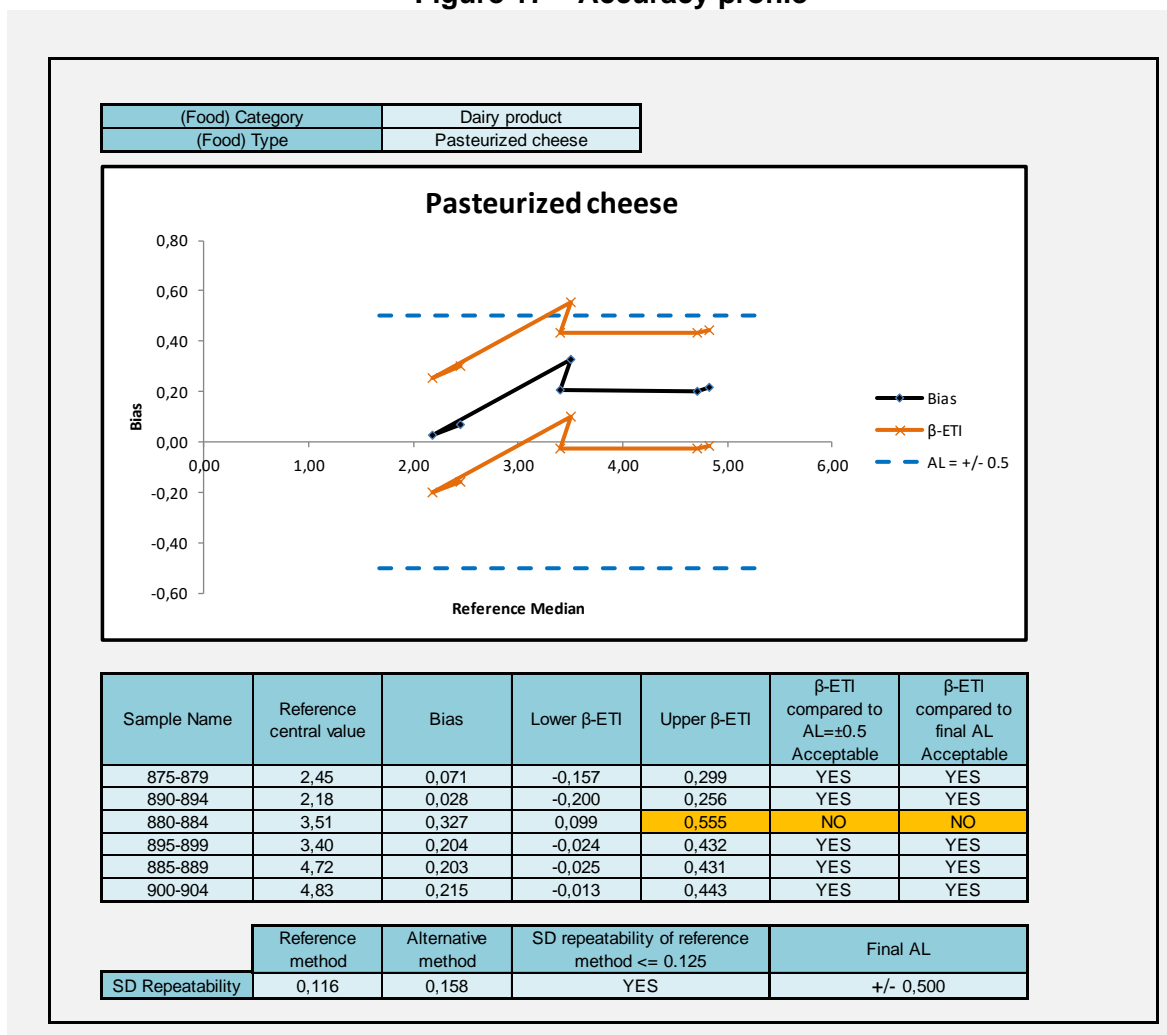
#### 3.2.2.2 Calculation and interpretation

The raw data are provided in **Appendix 12**. The summary tables (in log CFU/g) and calculations are provided in **Appendix 13**. The statistical results and the accuracy profiles are provided **Figure 17**.

The same results were obtained before and after storage of the TEMPO cards for 48h at 5°C ± 3°C.

The calculations will be done using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) ver 31-07-2018 available on <http://standards.iso.org/iso/16140>.

Figure 17 – Accuracy profile



The lower and upper  $\beta$ .ETI are within the acceptability limits for the low and high inoculation levels. For the intermediate inoculation level, the Upper  $\beta$ -ETI is above the AL fixed at  $\pm 0.5$  log for one batch (Upper  $\beta$ -ETI=0,555).

### 3.2.2.3 Conclusion

**The observed profiles meet the AL except for one batch inoculated at the intermediate level (Upper  $\beta$ -ETI>AL).**

### 3.2.3 Inclusivity study

This part was not tested again in agreement with AFNOR Technical committee.

### 3.2.4 Conclusion

The observed data and interpretation confirm the performances of the alternative method:

- **22 samples were tested in the relative trueness study**, which satisfied the required criteria for quantitative method comparison per ISO 16140-2; **this study confirms as well the possibility to store the TEMPO cards for 48h at 5°C ± 3°C.** Some discrepancies were observed between the ISO 16649-2 method and the TEMPO EC method but in some cases, the data observed with the TEMPO EC method fits better with the target inoculation level. This could be explained by the high selectivity of the TBX medium used in the ISO 16649-2 method.
- It is possible to store the TEMPO cards for 48h at 5°C ± 3°C before reading.
- The observed profiles meet the AL actually set at ± 0.5 Log CFU/g in the EN ISO 16140-2:2016 except for one batch inoculated at the intermediate level (Upper  $\beta$ -ETI= 0.555>AL=0.5).

### 3.3 Inter-laboratory study

*The aim of the inter-Laboratory study is to determine the variability of the results obtained in different laboratories using identical samples and to compare these results with those obtained in the methods comparison study.*

The results of the inter-laboratory Study run in 2005 were interpreted according to the EN ISO 16140-2:2016 standard using Excel spread sheet available at <http://standards.iso.org/iso/16140> (AP Calculation tool ILS (clause 6.2.3 Calculation summary and interpretations of data) ver 14.03.2016).

#### 3.3.1 Study organisation

The used matrix was pasteurized half-skimmed milk. Twelve laboratories participated to this study

#### 3.3.2 Experimental parameters control

##### 3.3.2.1 Strain stability

In order to verify the stability of the strain in the matrix, two samples per inoculation level were enumerated the day of inoculation and after one day storage at  $3^{\circ}\text{C} \pm 2^{\circ}\text{C}$  (See Table 17).

**Table 17 - *E. coli* 94 enumeration with the reference method (CFU/ml)**

	Level 1	Level 2	Level 3
Day 0	50	710	6 700
	100	850	7 200
Day 1	68	650	8 100
	59	740	8 800

No evolution was observed.

##### 3.3.2.2 Homogeneity of inoculation

This test was not run for the inter-laboratory study as it was not required in 2005 (inter-laboratory study run according to the ISO 16140:2003).

### 3.3.3 Results analysis

The raw data are given in **Appendix 14**.

#### 3.3.3.1 Results obtained by the expert Lab.

The results obtained by the expert Lab. are the following (See Table 18).

**Table 18 – Results obtained by the expert Lab.**

Inoculation level	Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
0	< 10	< 10	< 10	< 10
1	68	59	130	21
2	650	740	900	1 700
3	8 100	8 800	15 000	7 800

Targeted contamination levels were reached.

#### 3.3.3.2 Results obtained by the collaborators

A summary of the test results is given in Table 19 (CFU/g) and Table 20 (log CFU/g).

Lab E made an inversion between one sample from Level 0 (E5) and one sample from Level 1 (E2). This lab was not kept for data interpretation at Level 1.



**Table 19 - Summary of data (CFU/g)**

Labora- tories	Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
	Blank level				Low level				Medium level				High level			
Lab A	<10	<10	<10	<10	70	50	100	45	660	630	630	570	4300	3500	15000	6000
Lab B	<10	<10	<10	<10	80	85	89	100	530	740	810	830	4700	3700	15000	7800
Lab C	<10	<10	<10	<10	70	75	99	59	600	560	710	950	3400	3400	12000	11000
Lab D	<10	<10	<10	<10	80	45	86	45	730	690	530	900	4400	4100	12000	11000
Lab E	65	<10	<10	<10	<10	85	32	86	560	630	830	710	4800	4200	9100	6000
Lab F	<10	<10	<10	<10	73	68	68	83	550	610	690	1100	7000	7100	6700	9000
Lab G	<10	<10	<10	<10	35	80	68	33	650	690	930	570	4400	3000	12000	7800
Lab H	<10	<10	<10	<10	65	45	73	21	590	470	710	570	5000	6900	11000	9100
Lab I	<10	<10	<10	<10	45	65	71	71	700	510	830	1200	5300	4700	6100	7800
Lab J	<10	<10	<10	<10	95	30	100	130	530	570	730	1100	7100	13000	15000	12000
Lab K	<10	<10	<10	<10	55	70	71	33	360	490	790	810	4700	4600	7800	6000
Lab L	<10	<10	<10	<10	45	65	89	33	430	620	930	830	1700	2300	7800	6000

**Table 20 - Summary of data (log CFU/g)**

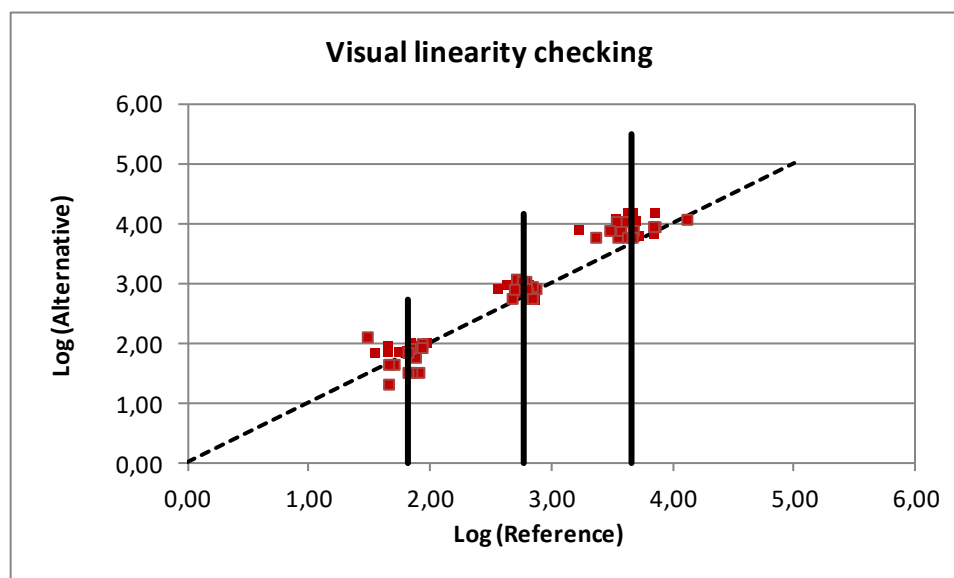
Labora- tories	Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
	Blank level				Low level				Medium level				High level			
Lab A	<1.00	<1.00	<1.00	<1.00	1.845	1.699	2.000	1.653	2.820	2.799	2.799	2.756	3.633	3.544	4.176	3.778
Lab B	<1.00	<1.00	<1.00	<1.00	1.903	1.929	1.949	2.000	2.724	2.869	2.908	2.919	3.672	3.568	4.176	3.892
Lab C	1.85	<1.60	1.70	<1.00	1.845	1.875	1.996	1.771	2.778	2.748	2.851	2.978	3.531	3.531	4.079	4.041
Lab D	<1.00	<1.00	<1.00	<1.00	1.903	1.653	1.934	1.653	2.863	2.839	2.724	2.954	3.643	3.613	4.079	4.041
Lab E	<1.00	<1.00	<1.00	<1.00	<1.000	1.929	1.505	1.934	2.748	2.799	2.919	2.851	3.681	3.623	3.959	3.778
Lab F	1.813	<1.00	<1.00	<1.00	1.863	1.833	1.833	1.919	2.740	2.785	2.839	3.041	3.845	3.851	3.826	3.954
Lab G	<1.00	<1.00	<1.00	<1.00	1.544	1.903	1.833	1.519	2.813	2.839	2.968	2.756	3.643	3.477	4.079	3.892
Lab H	<1.00	<1.00	<1.00	<1.00	1.813	1.653	1.863	1.322	2.771	2.672	2.851	2.756	3.699	3.839	4.041	3.959
Lab I	<1.00	<1.00	<1.00	<1.00	1.653	1.813	1.851	1.851	2.845	2.708	2.919	3.079	3.724	3.672	3.785	3.892
Lab J	<1.00	<1.00	<1.00	<1.00	1.978	1.477	2.000	2.114	2.724	2.756	2.863	3.041	3.851	4.114	4.176	4.079
Lab K	<1.00	<1.00	<1.00	<1.00	1.740	1.845	1.851	1.519	2.556	2.690	2.898	2.908	3.672	3.663	3.892	3.778
Lab L	<1.00	<1.00	<1.00	<1.00	1.653	1.813	1.949	1.519	2.633	2.792	2.968	2.919	3.230	3.362	3.892	3.778

### 3.3.4 Calculations and interpretation

#### 3.3.4.1 Visual linearity checking

The Figure 18 shows the data points after  $\log_{10}$  transformation. The visual inspection shows that the alternative method gives results, which are proportional to those of the reference method. The data are distributed closely to the first bisecting line.

**Figure 18 - Visual linearity checking**



#### 3.3.4.2 Accuracy profile calculation

Statistical calculations were done according to the Excel spreadsheet available on <http://standards.iso.org/ISO/16140>. A summary of the statistical test is provided in Table 21.

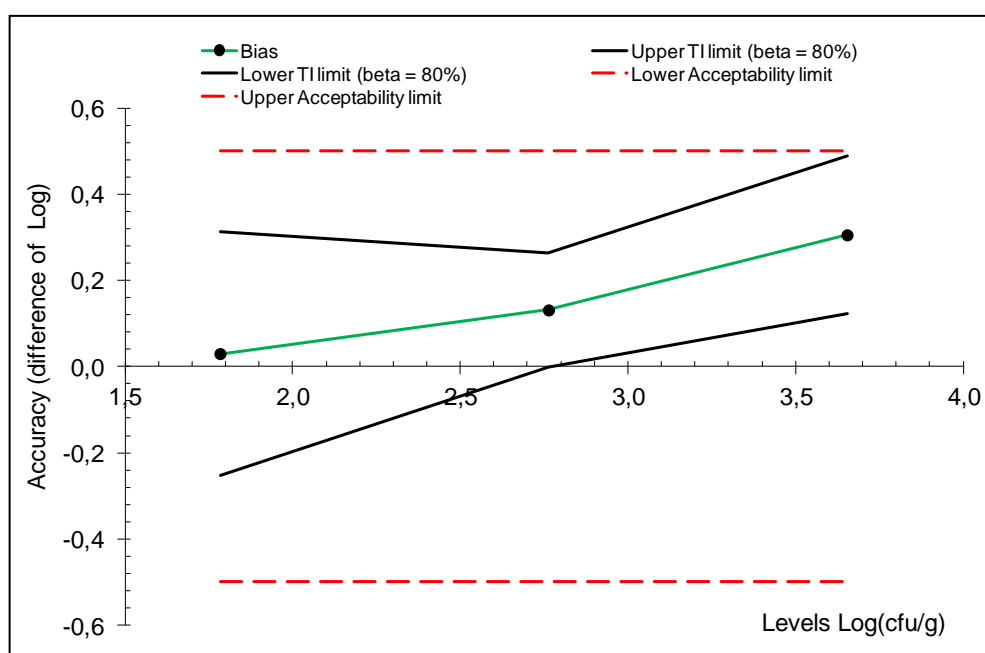
**Table 21 - Summary of the statistical test**

Accuracy profile							
Study Name	TEMPO EC						
Date	2005						
Coordinator	ADRIA						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	0.5	0.5	0.5				
Levels	Alternative method			Reference method			
	Low	Medium	High	Low	Medium	High	
<b>Target value</b>	<b>1.783</b>	<b>2.763</b>	<b>3.654</b>				
Number of participants (K)	11	12	12	11	12	12	
Average for alternative method	1.814	2.895	3.959	1.783	2.763	3.654	
Repeatability standard deviation (sr)	0.209	0.098	0.126	0.159	0.065	0.081	
Between-labs standard deviation (sL)	0.000	0.000	0.051	0.000	0.000	0.160	
Reproducibility standard deviation (sR)	0.209	0.098	0.136	0.159	0.076	0.180	
Corrected number of dof	20.952	22.957	22.246	20.952	21.036	13.501	
Coverage factor	1.353	1.347	1.352				
Interpolated Student t	1.323	1.320	1.321				
Tolerance interval standard deviation	0.2137	0.1001	0.1388				
Lower TI limit	1.531	2.763	3.776				
Upper TI limit	2.096	3.027	4.143				
<b>Bias</b>	<b>0.030</b>	<b>0.131</b>	<b>0.306</b>				
<b>Upper TI limit (beta = 80%)</b>	<b>-0.253</b>	<b>-0.001</b>	<b>0.123</b>				
<b>Lower TI limit (beta = 80%)</b>	<b>0.313</b>	<b>0.263</b>	<b>0.489</b>				
<b>Lower Acceptability limit</b>	<b>-0.50</b>	<b>-0.50</b>	<b>-0.50</b>				
<b>Upper Acceptability limit</b>	<b>0.50</b>	<b>0.50</b>	<b>0.50</b>				

Pooled repro standard dev = 0.145  
New acceptability limit (ALs) = 0.479

These values are collected in a graphical representation together with the acceptability limits (AL). This representation is given Figure 19.

**Figure 19**



It is observed that for all the levels, the tolerance interval limits of the alternative method are within the acceptable limits of 0.5 log.

**The results obtained with the alternative method are not statistically different than those obtained with the reference method.**

### 3.3.5 Conclusion

The quality assurance parameters were verified (i.e. inoculation homogeneity, targeted levels, strain stability, logistic conditions, analyses), confirming that the inter-laboratory study was conducted in appropriate conditions.

The data interpretations were done according to the EN ISO 16140-2:2016. For the three contamination levels, the alternative method is accepted as equivalent to the reference method.

## 4 GENERAL CONCLUSION

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The observed data and interpretation confirm the performances of the alternative method whatever the incubation temperature used for incubation of the TEMPO cards, 37°C for all food and pet food categories, 44°C for the dairy products category:

### Method comparison study

- > For the initial and extension studies concerning all food products and pet food categories with incubation of the TEMPO cards at 37°C:
  - 190 samples were tested in the relative trueness study providing 141 interpretable results by both the reference and the TEMPO EC methods, which clearly satisfied the required criteria for quantitative method comparison per ISO 16140-2;
  - The observed profiles are comprised within the AL actually set at 0.5 Log CFU/g in the EN ISO 16140-2:2016 for the 6 matrix/strain pairs in the range of contamination usually observed for *Escherichia coli* enumerations.
  - The inclusivity and exclusivity testing shows satisfying results.

- > **For the extension study concerning the dairy products category with incubation of the TEMPO cards at 44°C**
  - **22 interpretable data were obtained in the relative trueness study**, which satisfied the required criteria for quantitative method comparison per ISO 16140-2; **this study confirms as well the possibility to store the TEMPO cards for 48h at 5°C ± 3°C. Some discrepancies were observed between the ISO 16649-2 method and the TEMPO EC method but in some cases, the data observed with the TEMPO EC method fits better with the target inoculation level. This could be explained by the high selectivity of the TBX medium used in the ISO 16649-2 method.**
  - **It is possible to store the TEMPO cards for 48h at 5°C ± 3°C before reading.**
  - **The observed profiles meet the AL actually set at ± 0.5 Log CFU/g in the EN ISO 16140-2:2016 except for one batch inoculated at the intermediate level (Upper β-ETI= 0.555>AL=0.5).**

#### **Interlaboratory study**

- > **The quality assurance parameters were verified (i.e. inoculation homogeneity, targeted levels, strain stability, logistic conditions, analyses), confirming that the inter-laboratory study was conducted in appropriate conditions.**
  
- > **The data interpretations were done according to the EN ISO 16140-2:2016. For the three contamination levels, the alternative method is accepted as equivalent to the reference method.**

**Based on the results obtained for the method comparison study and the inter-laboratory study, the TEMPO® EC method is considered equivalent to the reference method.**

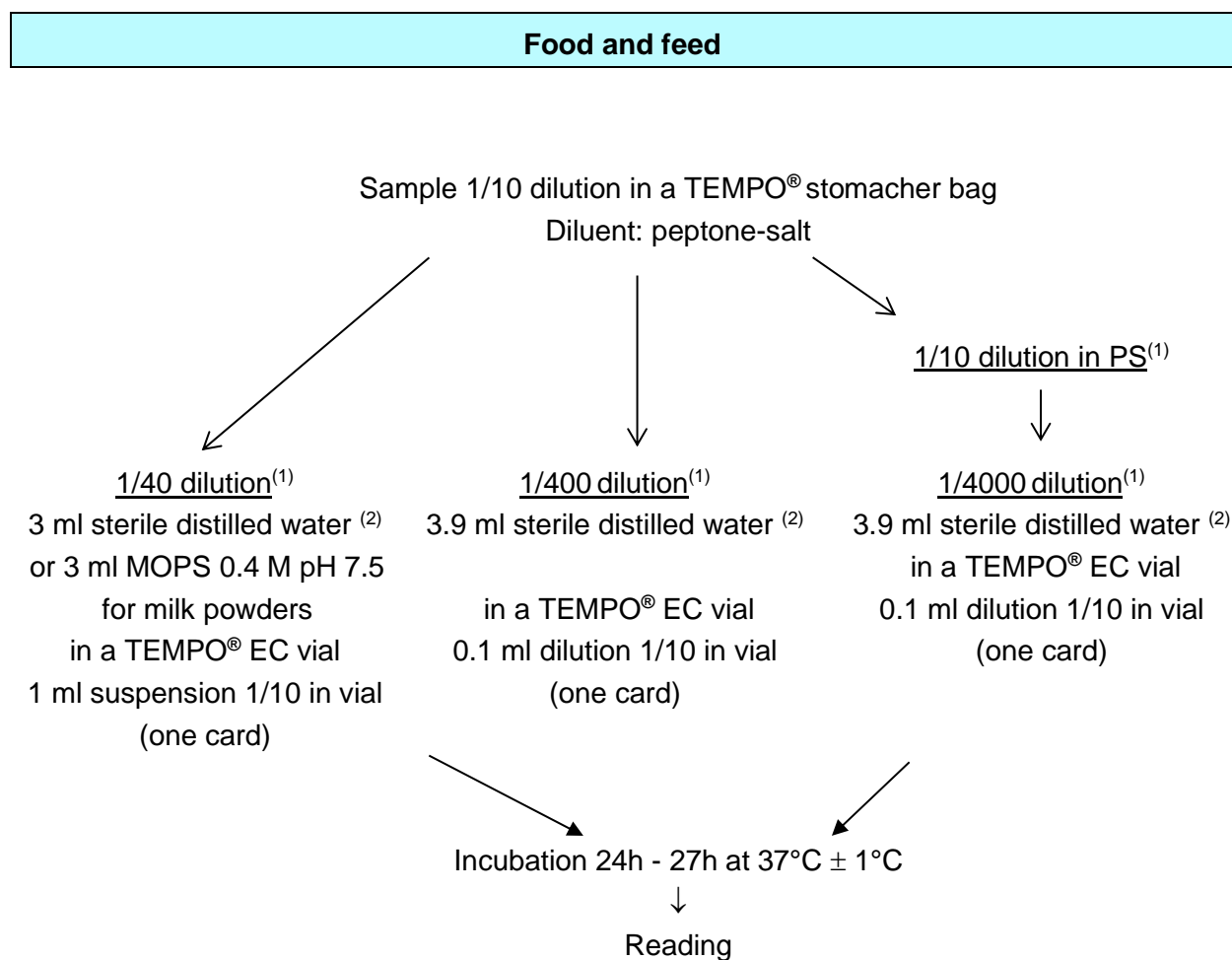
Quimper, 17 May 2023

Maryse RANNOU  
Project Manager  
Validation of Alternative methods  
*Food Safety & Quality*



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

## Appendix 1 - Flow diagram of the alternative method: TEMPO® EC



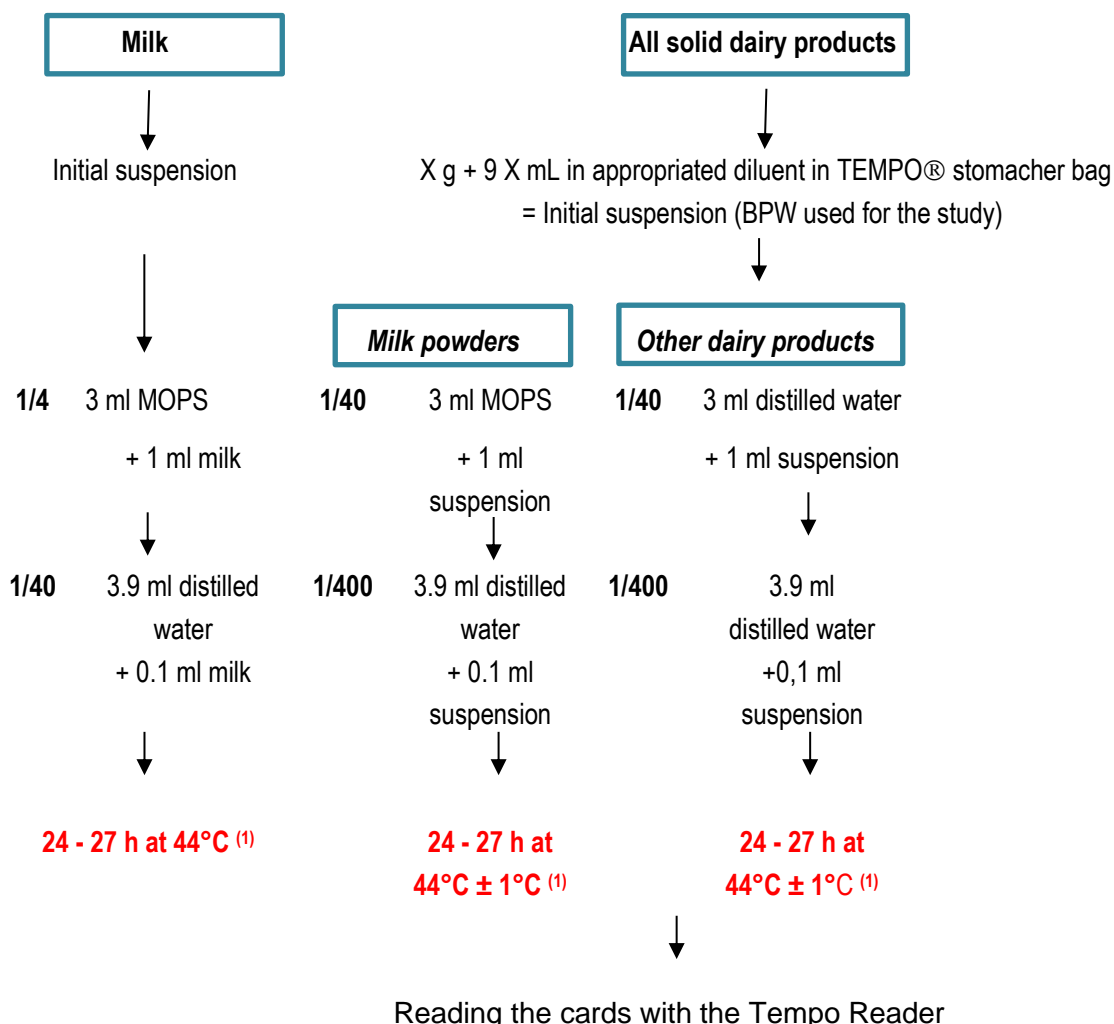
The 1/40 dilution allows 10 to 49 000 cfu/g enumeration

The 1/400 dilution allows 100 to 490 000 cfu/g enumeration

<sup>(1)</sup> Dilution in distilled water for all the products, except milk powders

<sup>(2)</sup> Dilutions mentioned here were tested during validations studies. Depending on the expected level of contamination and the nature of the product (liquid or solid), the dilution level can be adapted.

**Dairy products (Extension 2023)**

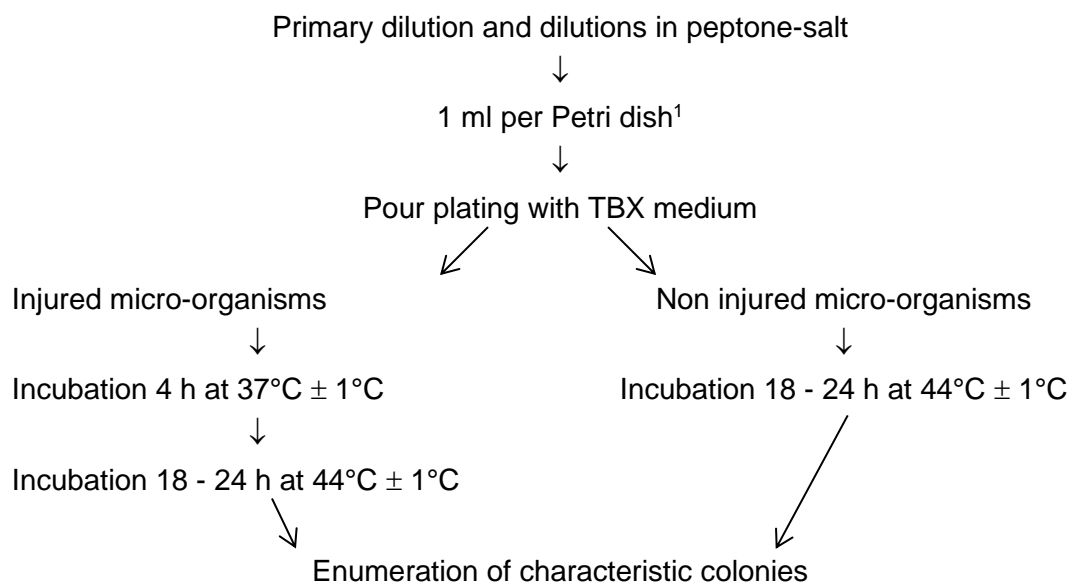


**Incubation 24h - 27h at 44°C ± 1°C for dairy products**  
**Possibility to store the TEMPO cards for 48 h at 5°C ± 3°C**

The 1/4 dilution allows 1 to 49 00 CFU/g enumeration  
 The 1/40 dilution allows 10 to 49 000 CFU/g enumeration  
 The 1/400 dilution allows 100 to 490 000 CFU/g enumeration

*Dilutions mentioned here were tested during validations studies. Depending on the expected level of contamination and the nature of the product (liquid or solid), the dilution level can be adapted.*

**Appendix 2 - Flow diagram of the reference method: the ISO 16649-2 (July 2001):  
Horizontal method for the enumeration of  $\beta$  glucuronidase positive *Escherichia coli* –  
Part 2: colony-count technique at 44°C using 5-bromo-4-chloro-3 indoly  $\beta$ -D-  
glucuronate**



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<sup>1</sup> Note that for the Validation Study, 2 Petri dishes were used per dilution for the analyses run in 2004 and 2006



**Appendix 3 - Artificial contamination of samples**

N° Sample	Analysis date	Product	Artificial contamination			
			Strain	Origin	Injury protocol	Injury measurement
5100	2010	Raw cow milk	<i>Escherichia coli</i> 14	Raw milk	4°C / 7 days	0.5
5101	2010	Raw cow milk	<i>Escherichia coli</i> 94	Cheese	4°C / 7 days	0.5
5102	2010	Raw cow milk	<i>Escherichia coli</i> E17	Raw milk	4°C / 7 days	0.5
5103	2010	Raw cow milk	<i>Escherichia coli</i> 14	Raw milk	4°C / 7 days	0.5
5104	2010	Raw cow milk	<i>Escherichia coli</i> 119	Raw milk	4°C / 7 days	0.5
5105	2010	Raw cow milk	<i>Escherichia coli</i> 94	Cheese	4°C / 7 days	0.5
240	2004	Ice cream (chocolate/pistachio)	Cross contamination with raw milk		Freezing	/
241	2004	Ice cream (red fruits)	Cross contamination with raw milk		Freezing	/
242	2004	Ice cream (vanilla)	Cross contamination with raw milk		Freezing	/
243	2004	Ice cream	Cross contamination with raw milk		Freezing	/
244	2004	Ready to reheat food	<i>Escherichia coli</i> 108	Ready to reheat (bouchée à la reine)	HT 56°C/15 min	0.44
245	2004	Ready to reheat food	<i>Escherichia coli</i> 108	Ready to reheat (bouchée à la reine)	HT 56°C/15 min	0.44
250	2004	Pellets for kitten	<i>Escherichia coli</i> 144	Paella	HT 56°C/15 min	0.77
251	2004	Pellets	<i>Escherichia coli</i> 144	Paella	HT 56°C/15 min	0.77
252	2004	Pellets	<i>Escherichia coli</i> 144	Paella	HT 56°C/15 min	0.77
253	2004	Frozen ready to reheat vegetables	<i>Escherichia coli</i> 19	Sliced carrots	-20°C / 1 month	1.45
254	2004	Frozen ready to reheat vegetables	<i>Escherichia coli</i> 19	Sliced carrots	-20°C / 1 month	1.45
255	2004	Frozen ready to reheat vegetables	<i>Escherichia coli</i> 19	Sliced carrots	-20°C / 1 month	1.45
256	2004	Raw sea almonds	Contamination by soaking			
257	2004	Raw clams	Contamination by soaking			
258	2004	Raw oysters	Contamination by soaking			
259	2004	Carrots	Contamination by soaking			

N° Sample	Analysis date	Product	Artificial contamination			
			Strain	Origin	Injury protocol	Injury measurement
260	2004	Turnips	Contamination by soaking			
261	2004	Green cabbage	Contamination by soaking			
264	2004	Ice cream (vanilla/pistachio)	Cross contamination with raw milk		Freezing	/
271	2004	Sausage for dog	<i>Escherichia coli</i> 108	Ready to reheat (bouchée à la reine)	HT 56°C/15 min	1.09
272	2004	Sausage for dog	<i>Escherichia coli</i> 123	Veal liver	HT 56°C/15 min	0.39
273	2004	Pâté for dog	<i>Escherichia coli</i> 21	Cured meat	HT 56°C/15 min	0.47
274	2004	Pâté for dog	<i>Escherichia coli</i> 13	Ground beef	HT 56°C/15 min	0.78
275	2004	Frozen ground beef	<i>Escherichia coli</i> 13	Ground beef	-20°C / 2 months	3.5
276	2004	Frozen poultry meat	<i>Escherichia coli</i> 21	Cured meat	-20°C / 2 months	1.49
277	2004	Frozen pork meat	<i>Escherichia coli</i> 123	Veal liver	-20°C / 2 months	1.84
278	2004	Cooked mussels	<i>Escherichia coli</i> Ad228	Fish	Osmotic stress (35 °/∞ NaCl)	0.36
279	2004	Tuna	<i>Escherichia coli</i> 93	Ready to reheat fish	Osmotic stress (35 °/∞ NaCl)	>3.15
280	2004	Fish fillet	<i>Escherichia coli</i> 93	Ready to reheat fish	Osmotic stress (35 °/∞ NaCl)	>3.15
281	2004	Grated carrots	Cross contamination with water			
282	2004	Cabbage	Cross contamination with water			
283	2004	Mixed salad	Cross contamination with water			
293	2004	Dried apple	Contact with desiccated bacteria suspension and storage 24h at ambient temperature			
294	2004	Dried apple	Contact with desiccated bacteria suspension and storage 24h at ambient temperature			
2504	2006	Infant formula milk powder	<i>Escherichia coli</i> 16	Raw milk	HT 55°C/30 min	1.6
2505	2006	Infant formula milk powder	<i>Escherichia coli</i> 16	Raw milk	HT 55°C/30 min	1.6
2506	2006	Infant formula milk powder	<i>Escherichia coli</i> 16	Raw milk	HT 55°C/30 min	1.6
2507	2006	Infant formula milk powder	<i>Escherichia coli</i> 16	Raw milk	HT 55°C/30 min	1.6
2508	2006	Infant formula milk powder	<i>Escherichia coli</i> 16	Raw milk	HT 55°C/30 min	1.6
2509	2006	Infant formula milk powder	<i>Escherichia coli</i> 14	Raw milk	HT 55°C/30 min	0.6
2510	2006	Infant formula milk powder	<i>Escherichia coli</i> 14	Raw milk	HT 55°C/30 min	0.6
2511	2006	Infant formula milk powder	<i>Escherichia coli</i> 14	Raw milk	HT 55°C/30 min	0.6
2512	2006	Infant formula milk powder	<i>Escherichia coli</i> 14	Raw milk	HT 55°C/30 min	0.6
2513	2006	Milk powder	<i>Escherichia coli</i> 14	Raw milk	HT 55°C/30 min	0.6

N° Sample	Analysis date	Product	Artificial contamination			
			Strain	Origin	Injury protocol	Injury measurement
2514	2006	Infant formula milk powder	<i>Escherichia coli</i> 15	Raw milk	HT 55°C/30 min	1.8
2515	2006	Infant formula milk powder	<i>Escherichia coli</i> 15	Raw milk	HT 55°C/30 min	1.8
2516	2006	Infant formula milk powder	<i>Escherichia coli</i> 15	Raw milk	HT 55°C/30 min	1.8
2517	2006	Infant formula milk powder	<i>Escherichia coli</i> 15	Raw milk	HT 55°C/30 min	1.8
2518	2006	Infant formula milk powder	<i>Escherichia coli</i> 15	Raw milk	HT 55°C/30 min	1.8
2519	2006	Infant formula milk powder	<i>Escherichia coli</i> 119	Raw milk	HT 55°C/30 min	1.6
2520	2006	Infant formula milk powder	<i>Escherichia coli</i> 119	Raw milk	HT 55°C/30 min	1.6
2521	2006	Infant formula milk powder	<i>Escherichia coli</i> 119	Raw milk	HT 55°C/30 min	1.6
2522	2006	Infant formula milk powder	<i>Escherichia coli</i> 119	Raw milk	HT 55°C/30 min	1.6
2523	2006	Infant formula milk powder	<i>Escherichia coli</i> 119	Raw milk	HT 55°C/30 min	1.6
2524	2006	Infant formula milk powder	<i>Escherichia coli</i> 97	Raw milk	HT 55°C/30 min	7.7
2525	2006	Infant formula milk powder	<i>Escherichia coli</i> 97	Raw milk	HT 55°C/30 min	7.7
2546	2006	Infant formula milk powder	<i>Escherichia coli</i> 94	Raw milk	HT 55°C/30 min	0.6
2547	2006	Infant formula milk powder	<i>Escherichia coli</i> 94	Raw milk	HT 55°C/30 min	0.6
2548	2006	Infant formula milk powder	<i>Escherichia coli</i> E17	Raw milk	HT 55°C/30 min	1.1
2549	2006	Infant formula milk powder	<i>Escherichia coli</i> E17	Raw milk	HT 55°C/30 min	1.1
2550	2006	Infant formula milk powder	<i>Escherichia coli</i> 121	Raw milk	HT 55°C/30 min	1.0
2551	2006	Infant formula milk powder	<i>Escherichia coli</i> 121	Raw milk	HT 55°C/30 min	1.0
2552	2006	Infant formula milk powder	<i>Escherichia coli</i> 121	Raw milk	HT 55°C/30 min	1.0
2553	2006	Infant formula milk powder	<i>Escherichia coli</i> 121	Raw milk	HT 55°C/30 min	1.0
2058	2011	Ratatouille	<i>Escherichia coli</i> 19	Sliced carrots	Seeding HT 56°C/15 min	/
2059	2011	Chilli con carne	<i>Escherichia coli</i> 144	Paella	HT 56°C/15 min	/
2060	2011	Ready to cook vegetables	<i>Escherichia coli</i> 144	Paella	Seeding HT 56°C/15 min	/
2061	2011	Ready to eat Chinese food	<i>Escherichia coli</i> 93	Ready to reheat fish	Seeding HT 56°C/15 min	/
2062	2011	Pastry	<i>Escherichia coli</i> Ad 222	Egg product	Seeding HT 56°C/15 min	/
2063	2011	Pastry	<i>Escherichia coli</i> 142	Egg product	Seeding HT 56°C/15 min	/
2064	2011	Pastry	<i>Escherichia coli</i> Ad 222	Egg product	Seeding HT 56°C/15 min	/
2065	2011	Pork nem	<i>Escherichia coli</i> 108	Ready to reheat meal	Seeding HT 56°C/15 min	/

N° Sample	Analysis date	Product	Artificial contamination			
			Strain	Origin	Injury protocol	Injury measurement
2066	2011	Chinese ravioli with pork	<i>Escherichia coli</i> 108	Ready to reheat meal	Seeding HT 56°C/15 min	/
2120	2011	Frozen ratatouille	<i>Escherichia coli</i> 19	Sliced carrots	Seeding -20°C / 8 Days	/
2121	2011	Ready to eat salad	<i>Escherichia coli</i> 19	Sliced carrots	Seeding -20°C / 8 Days	/
2122	2011	Smoked salmon sandwich	<i>Escherichia coli</i> 93	Ready to reheat fish	Seeding -20°C / 8 Days	/
2123	2011	Tabbouleh	<i>Escherichia coli</i> 108	Ready to reheat meal	Seeding -20°C / 8 Days	/
2150	2011	Ready to eat pasta salad	<i>Escherichia coli</i> Ad 222	Egg product	Seeding HT 10min 56°C	/
2151	2011	Sandwich ham egg crudités	<i>Escherichia coli</i> Ad 222	Egg product	4°C / 1 Day	/
2152	2011	Smoked sausages	<i>Escherichia coli</i> 21	Raw delicatessen	Seeding HT 10min 56°C	/
2153	2011	Smoked sausages	<i>Escherichia coli</i> 21	Raw delicatessen	Seeding HT 10min 56°C	/
2183	2011	Frozen leeks quiche	<i>Escherichia coli</i> 142	Egg product	Seeding -20°C / 6 days	/
2184	2011	Sandwich chicken and egg	<i>Escherichia coli</i> 142	Egg product	Seeding 4°C / 6 days	/
2185	2011	Sausages	<i>Escherichia coli</i> 6	Sausage	Seeding 4°C / 6 days	/
2186	2011	Poultry meat	<i>Escherichia coli</i> 96	Turkey meat	Seeding 4°C / 6 days	/
2363	2011	Salad carrots celery	<i>Escherichia coli</i> 19	Sliced carrots	Seeding 4°C / 7 days	/
2364	2011	Celery remoulade	<i>Escherichia coli</i> 144	Paella	Seeding 4°C / 7 days	/
2365	2011	Mixed vegetables	<i>Escherichia coli</i> 144	Paella	Seeding HT 56°C/15 min	/
2366	2011	Mixed vegetables	<i>Escherichia coli</i> 142	Egg products	Seeding HT 56°C/10 min	/
2368	2011	Sandwich ham egg tomatoes	<i>Escherichia coli</i> 101	Pork	Seeding HT 56°C/10 min	/
2369	2011	Cooked ham	<i>Escherichia coli</i> 101	Pork	Seeding HT 56°C/10 min	/
2470	2011	Garlic sausage	<i>Escherichia coli</i> 1	Pork	Seeding HT 56°C/10 min	/
2472	2011	Chorizo	<i>Escherichia coli</i> 1	Pork	Seeding HT 56°C/10 min	/
2473	2011	Garlic sausage	<i>Escherichia coli</i> 1	Pork	Seeding HT 56°C/10 min	/
2475	2011	Dry ham	<i>Escherichia coli</i> 1	Pork	Seeding 4°C / 7 days	/
2476	2011	Bacon	<i>Escherichia coli</i> 3A	Pork	Seeding 4°C / 7 days	/
2478	2011	Sliced bacon	<i>Escherichia coli</i> 1	Pork	Seeding 4°C / 7 days	/
2479	2011	Ham	<i>Escherichia coli</i> 21	Pork	Seeding 4°C / 7 days	/
2480	2011	Sandwich chicken mayonnaise	<i>Escherichia coli</i> Ad 218	Poultry	Seeding 4°C / 7 days	/

N° Sample	Analysis date	Product	Artificial contamination			
			Strain	Origin	Injury protocol	Injury measurement
2481	2011	<b>Sandwich chicken mayonnaise</b>	<i>Escherichia coli</i> Ad 218	Poultry	Seeding 4°C / 7 days	/
2482	2011	<b>Ready to eat salad</b>	<i>Escherichia coli</i> 108	Ready to reheat	Seeding HT 10min 56°C	/
7202	2016	<b>Tabbouleh</b>	<i>Escherichia coli</i> 19	Grated carrots	Seeding 2-8°C 48h	/
7203	2016	<b>Grated carrots</b>	<i>Escherichia coli</i> 19	Grated carrots	Seeding 2-8°C 48h	/
7204	2016	<b>Cucumbers with cream</b>	<i>Escherichia coli</i> 19	Grated carrots	Seeding 2-8°C 48h	/
7205	2016	<b>Sausage for dog</b>	<i>Escherichia coli</i> 96	Poultry meat	HT 56°C/10 min	0.46
7206	2016	<b>Pellets for cats</b>	<i>Escherichia coli</i> 96	Poultry meat	HT 56°C/10 min	0.46
7207	2016	<b>Pellets for cats</b>	<i>Escherichia coli</i> 96	Poultry meat	HT 56°C/10 min	0.46
7453	2016	<b>Raw shrimps</b>	<i>Escherichia coli</i> Ad228	Fish	Seeding 2-8°C 48h	/
7454	2016	<b>Shrimps with spicy sauce</b>	<i>Escherichia coli</i> Ad1401	Sea water	Seeding 2-8°C 48h	/
7455	2016	<b>Cod fritters</b>	<i>Escherichia coli</i> Ad1403	Sea water	Seeding 2-8°C 48h	/
7456	2016	<b>Smoked trout</b>	<i>Escherichia coli</i> Ad1401	Sea water	Seeding 2-8°C 48h	/
7457	2016	<b>Smoked mackerel</b>	<i>Escherichia coli</i> Ad1403	Sea water	Seeding 2-8°C 48h	/
7458	2016	<b>Zucchini</b>	<i>Escherichia coli</i> Ad1396	Tap water	Seeding 2-8°C 48h	/
7459	2016	<b>Raw meat for animals</b>	<i>Escherichia coli</i> 13	Ground beef	Seeding 2-8°C 48h	/
7460	2016	<b>Raw meat for animals</b>	<i>Escherichia coli</i> 123	Veal liver	Seeding 2-8°C 48h	/
7461	2016	<b>Pâté for dog</b>	<i>Escherichia coli</i> Ad1828	Beef	HT 56°C/10 min	0.63
7462	2016	<b>Pellets for dog</b>	<i>Escherichia coli</i> Ad1828	Beef	HT 56°C/10 min	0.63

Appendix 4 – Relative trueness study: raw data (Initial validation study and extension studies)

READY TO EAT AND READY TO REHEAT																							Category	Type	
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*								Alternative method: TEMPO EC													
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1			Replicate 2			Replicate 1	Replicate 2	Mean				
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g		
2062	2011	Pastry	10	21				220		2,34		2,34	160	210		160				2,20		2,20	1	a	
			100	3																					
2063	2011	Pastry	100	35				4300		3,63		3,63	3100	<100		3100				3,49		3,49	1	a	
			1000	12																					
2064	2011	Pastry	1000	50				49000		4,69		4,69	490000	40000		40000				4,60		4,60	1	a	
			10000	4																					
2122	2011	Smoked salmon sandwich	100	0				<10		<1,00		<1,00	<10	<100		<10				<1,00		<1,00	1	a	
			1000	0																					
2150	2011	Ready to eat pasta salad	10	21				240		2,38		2,38	140	210		140				2,15		2,15	1	a	
			100	5																					
2151	2011	Sandwich ham egg crudités	100	50				5100		3,71		3,71	3100	2800		3100				3,49		3,49	1	a	
			1000	6																					
2184	2011	Sandwich chicken and egg	100	50				4700		3,67		3,67	3400	3600		3400				3,53		3,53	1	a	
			1000	2																					
2368	2011	Sandwich ham egg tomatoes	10	>150				4500		3,65		3,65	1900	1300		1900				3,28		3,28	1	a	
			100	45				N'																	
2480	2011	Sandwich chicken mayonnaise	10	20				190		2,28		2,28	220	100		220				2,34		2,34	1	a	
			100	1																					
2481	2011	Sandwich chicken mayonnaise	10	14				160		2,20		2,20	160	650		160				2,20		2,20	1	a	
			100	4																					
244	2004	Ready to reheat food	10	106	118	99	130	1100	1100	3,04	3,04	3,04	6000	5200		6000	6000	3300		6000	3,78	3,78	3,78	1	b
			100	10	9	6	6																		
277	2004	Frozen pork meat	100	39	46	58	42	4300	5100	3,63	3,71	3,67	21000	12000		21000	25000	21000		25000	4,32	4,40	4,36	1	b
			1000	6	4	7	5																		
2059	2011	Chilli con carne	10	>150				1900		3,28		3,28	2100	1100		2100				3,32		3,32	1	b	
			100	19				N'																	
2061	2011	Ready to eat Chinese food	10	0				<10		<1,00		<1,00	<10	<100		<10				<1,00		<1,00	1	b	
			100	0																					
2065	2011	Pork nem	100	71				7000		3,85		3,85	6000	990		6000				3,78		3,78	1	b	
			1000	6																					
2066	2011	Chinese ravioli with pork	10	104				1100		3,04		3,04	260	<100		260				2,41		2,41	1	b	
			100	16																					
2183	2011	Frozen leeks quiche	1000	69				65000		4,81		4,81	>49000	60000		60000				4,78		4,78	1	b	
			10000	3																					
2482	2011	Ready to eat salad	10	96				940		2,97		2,97	460	440		460				2,66		2,66	1	b	
			100	7																					
2369	2011	Cooked ham	10	>150				1800		3,26		3,26	240	210		240				2,38		2,38	1	c	
			100	18				N'																	
2470	2011	Garlic sausage	10	82				820		2,91		2,91	570	210		570				2,76		2,76	1	c	
			100	8																					
2472	2011	Chorizo	100	38				3700		3,57		3,57	2900	1200		2900				3,46		3,46	1	c	
			1000	3																					
2473	2011	Garlic sausage	100	66				6700		3,83		3,83	6800	5100		6800				3,83		3,83	1	c	
			1000	8																					



MEAT PRODUCTS																								Category	Type
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*								Alternative method: TEMPO EC													
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean log CFU/g	Replicate 1			Replicate 2			Replicate 1 log CFU/g	Replicate 2 log CFU/g	Mean log CFU/g				
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g		d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result					
1911	2011	Veal roulade	10	114				1100		3,04		3,04	810	210		810				2,91		2,91	2	a	
			100	5																					
1912	2011	Veal roulade	10	93				910		2,96		2,96	950	860		950				2,98		2,98	2	a	
			100	7																					
1918	2011	Pork meat	100	100				10000		4,00		4,00	9200	9100		9200				3,96		3,96	2	a	
			1000	10																					
1919	2011	Pork meat	1000	52				53000		4,72		4,72		44000	63000	44000				4,64		4,64	2	a	
			10000	6																					
275	2004	Frozen ground beef	10	53	71	62	64	620	600	2,79	2,78	2,79	1500	320		1500	830	1100		830	3,18	2,92	3,05	2	b
			100	7	6	4	2																		
276	2004	Frozen poultry meat	100	25	15	27	25	2000	2600	3,30	3,41	3,36	6800	3000		6800	5000	4800		5000	3,83	3,70	3,77	2	b
			1000	1	3	2	4																		
1866	2011	Poultry meat	10	54				560		2,75		2,75	900	710		900				2,95		2,95	2	b	
			100	7																					
1867	2011	Poultry meat	10	47				510		2,71		2,71	220	450		220				2,34		2,34	2	b	
			100	9																					
1868	2011	Poultry meat	100	60				5900		3,77		3,77	6000	5700		6000				3,78		3,78	2	b	
			1000	5																					
1913	2011	Poultry meat	100	57				5400		3,73		3,73	7800	6300		7800				3,89		3,89	2	b	
			1000	2																					
1915	2011	Poultry meat	1000	19				17000		4,23		4,23	21000	29000		21000				4,32		4,32	2	b	
			10000	0																					
1916	2011	Poultry meat	10	135				1400		3,15		3,15	930	570		930				2,97		2,97	2	b	
			100	14																					
1917	2011	Poultry meat	10	6				55 Ne		1,74		1,74	280	100		280				2,45		2,45	2	b	
			100	0																					
2186	2011	Poultry meat	10	17				160		2,20		2,20	160	330		160				2,20		2,20	2	b	
			100	1																					
1865	2011	Frozen sausage	10	1				10		1,00		1,00*	<10	<100		<10				<1,00		<1,00	2	c	
			100	1																					
1869	2011	Sausage	10	99				990		3,00		3,00	810	890		810				2,91		2,91	2	c	
			100	10																					
1870	2011	Sausage	10	81				800		2,90		2,90	730	830		730				2,86		2,86	2	c	
			100	7																					
1871	2011	Smoked sausage	10	50				490		2,69		2,69	430	210		430				2,63		2,63	2	c	
			100	4																					
2152	2011	Smoked sausages	10	4				40 Ne		1,60		1,60	33	100		33				1,52		1,52	2	c	
			100	0																					
2153	2011	Smoked sausages	100	43				4200		3,62		3,62	3400	3600		3400				3,53		3,53	2	c	
			1000	3																					
2185	2011	Sausages	10	16				160		2,20		2,20	45	<100		45				1,65		1,65	2	c	
			100	2																					
2475	2011	Dry ham	10	7				70 Ne		1,85		1,85	59	100		59				1,77		1,77	2	c	
			100	0																					
2476	2011	Bacon	10	5				50 Ne		1,70		1,70	89	100		89				1,95		1,95	2	c	
			100	1																					
2478	2011	Sliced bacon	100	>150				21000 N'		4,32		4,32	17000	29000		17000				4,23		4,23	2	c	
			1000	21																					
2479	2011	Ham	1000	62				62000		4,79		4,79	>49000	91000		91000				4,96		4,96	2	c	
			10000	6																					

DAIRY PRODUCTS																							Category	Type	
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC												
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1			Replicate 2			Replicate 1	Replicate 2	Mean				
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g		
161	2004	Liver	1000	34	22	26	20	27000	25000	4,43	4,40	4,41	/	67000		67000	/	50000		50000	4,83	4,70	4,76	3	a
			10000	3	1	2	6																		
162	2004	Veal meat	10	6	15	5	9	95	70	1,98	1,85	1,91	71	<100		71	170	<100		170	1,85	2,23	2,04	3	a
			100	0	0	1	0																		
193	2004	Raw cow milk cheese	10	3	3	2	2	30	20	1,48	1,30	1,39*	130	210		130	45	100		45	2,11	1,65	1,88	3	a
			100	0	0	0	0																		
194	2004	Raw goat milk cheese	10	12	11	15	10	120	140	2,08	2,15	2,11	2900	1900		2900	3400	2600		3400	3,46	3,53	3,50	3	a
			100	0	2	2	3																		
195	2004	Raw cow milk cheese	10	6	2	5	7	40	60	1,60	1,78	1,69	180	100		180	220	200		220	2,26	2,34	2,30	3	a
			100	0	0	0	0																		
240	2004	Ice cream (chocolate/pistachio)	10	81	77	92	86	800	890	2,90	2,95	2,93	730	1200		730	620	730		620	2,86	2,79	2,83	3	a
			100	10	8	7	9																		
241	2004	Ice cream (red fruits)	10	0	0	1	0	<10	5*	<1,00	0,7*	<1,00	140	100		140	120	100		120	2,15	2,08	2,11	3	a
			100	0	0	0	0																		
242	2004	Ice cream (vanilla)	10	82	89	98	113	860	1000	2,93	3,00	2,97	440	1500		440	730	810		730	2,64	2,86	2,75	3	a
			100	7	11	8	7																		
243	2004	Ice cream	10	107	138	113	124	1300	1200	3,11	3,08	3,10	1400	860		1400	1100	1300		1100	3,15	3,04	3,09	3	a
			100	19	11	18	15																		
264	2004	Ice cream (vanilla/pistachio)	100	>150	>167	>150	>150	21000	20000	4,32	4,30	4,31	37000	37000		37000	30000	37000		30000	4,57	4,48	4,52	3	a
			1000	29	13	22	17																		
160	2004	Ready to reheat food	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	3	b
			100	0	0	0	0																		
2504	2006	Infant formula milk powder	10	4	1	1	0	25	5	1,40	0,70	1,05*	<10	<100		<10	10	<100		10	<1,00	1,00	1,00	3	b
			100	0	0	0	0																		
2505	2006	Infant formula milk powder	10	19	17	14	23	180	180	2,26	2,26	2,26	21	<100		21	89	<100		89	1,32	1,95	1,64	3	b
			100	1	2	2	0																		
2506	2006	Infant formula milk powder	100	9	9	11	8	900	950	2,95	2,98	2,97	810	1200		810	1000	590		1000	2,91	3,00	2,95	3	b
			1000	1	0	0	0																		
2507	2006	Infant formula milk powder	1000	14	9	15	7	12000	11000	4,08	4,04	4,06	9100	31000		9100	12000	9300		12000	3,96	4,08	4,02	3	b
			10000	2	1	3	1																		
2508	2006	Infant formula milk powder	1000	15	9	7	11	11000	8700	4,04	3,94	3,99	9000	12000		9000	11000	13000		11000	3,95	4,04	4,00	3	b
			10000	0	0	1	0																		
2509	2006	Infant formula milk powder	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10			<10	<10			<10	<1,00	<1,00	<1,00	3	b
			100	0	0	0	0																		
2510	2006	Infant formula milk powder	10	1	1	0	1	10	5	1,00	0,70	0,85*	<10			<10	<10			<10	<1,00	<1,00	<1,00	3	b
			100	0	0	0	0																		
2511	2006	Infant formula milk powder	10	21	32	22	23	250	240	2,40	2,38	2,39	<10			<10	<10			<10	<1,00	<1,00	<1,00	3	b
			100	1	1	4	4																		
2512	2006	Infant formula milk powder	100	34	27	33	21	2900	2800	3,46	3,45	3,45	2200			2200	No result (error 19)			No result	3,34	No result	No result	3	b
			1000	1	1	5	2																		
2513	2006	Milk powder	100	44	36	36	26	3800	3300	3,58	3,52	3,55	3100			3100	1900			1900	3,49	3,28	3,39	3	b
			1000	1	2	4	6																		
2514	2006	Infant formula milk powder	10	4	4	5	4	40	45	1,60	1,65	1,63	120			120	59			59	2,08	1,77	1,93	3	b
			100	1	0	1	0																		
2515	2006	Infant formula milk powder	10	18	14	12	19	170	160	2,23	2,20	2,22	190			190	160			160	2,28	2,20	2,24	3	b
			100	2	3	1	3																		
2516	2006	Infant formula milk powder	10	59	69	76	81	660	790	2,82	2,90	2,86	730			730	830			830	2,86	2,92	2,89	3	b
			100	7	10	10	7																		
2517	2006	Infant formula milk powder	10	149	144	146	133	1500	1400	3,18	3,15	3,16	1900			1900	1700			1700	3,28	3,23	3,25	3	b
			100	13	18	13	20																		

\* Analyses performed according to the COFRAC accreditation



DAIRY PRODUCTS																							Category	Type		
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC													
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1			Replicate 2			Replicate 1	Replicate 2	Mean					
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g			
2518	2006	Infant formula milk powder	100	13	12	17	13	1300	1500	3,11	3,18	3,15	3100			3100	3700			3700	3,49	3,57	3,53	3	b	
			1000	2	1	0	2																			
2519	2006	Infant formula milk powder	100	24	17	26	25	2200	2500	3,34	3,40	3,37	3400	2600		3400	4100	3300			4100	3,53	3,61	3,57	3	b
			1000	2	5	3	1																			
2520	2006	Infant formula milk powder	1000	31	24	27	23	26000	24000	4,41	4,38	4,40	14000	44000		14000	No result (error 19)	55000			55000	4,15	4,74	4,44	3	b
			10000	2	1	1	2																			
2521	2006	Infant formula milk powder	1000	21	20	30	23	20000	25000	4,30	4,40	4,35	30000	37000		30000	49000	24000			49000	4,48	4,69	4,58	3	b
			10000	2	2	1	0																			
2522	2006	Infant formula milk powder	1000	39	30	46	50	35000	45000	4,54	4,65	4,60	No result (error 19)	45000		45000	No result (error 19)	68000			68000	4,65	4,83	4,74	3	b
			10000	4	5	2	2																			
2523	2006	Infant formula milk powder	1000	112	104	94	103	107000	100000	5,03	5,00	5,01	>49000	91000		91000	>49000	120000			120000	4,96	5,08	5,02	3	b
			10000	12	8	15	9																			
2524	2006	Infant formula milk powder	10	>150	>150	>150	>150	4900	4900	3,69	3,69	3,69	9100			9100	6800			6800	3,96	3,83	3,90	3	b	
			100	46	51	53	45																			N'
2525	2006	Infant formula milk powder	10	>150	>150	>150	>150	8500	8000	3,93	3,90	3,92	2400			2400	7400			7400	3,38	3,87	3,62	3	b	
			100	85	84	72	88																			N'
2546	2006	Infant formula milk powder	10	1	1	6	6	10	60	1,00	1,78	1,39*	<10			<10	10			10	<1,00	1,00	1,00	3	b	
			100	0	0	0	0																			Ne
2547	2006	Infant formula milk powder	10	3	6	2	3	45	25	1,65	1,40	1,53*	10			10	21			21	1,00	1,32	1,16	3	b	
			100	0	0	0	0																			Ne
2548	2006	Infant formula milk powder	10	10	10	11	5	100	80	2,00	1,90	1,95	100			100	100			100	2,00	2,00	2,00	3	b	
			100	0	0	2	0																			Ne
2549	2006	Infant formula milk powder	10	10	13	7	11	120	90	2,08	1,95	2,02	120			120	99			99	2,08	2,00	2,04	3	b	
			100	0	1	2	2																			Ne
2550	2006	Infant formula milk powder	10	17	29	15	24	220	190	2,34	2,28	2,31	490	310		490	330	100			330	2,69	2,52	2,60	3	b
			100	1	2	1	2																			
2551	2006	Infant formula milk powder	10	11	20	14	17	160	160	2,20	2,20	2,20	300	440		300	380	100			380	2,48	2,58	2,53	3	b
			100	2	3	0	3																			
2552	2006	Infant formula milk powder	100	10	9	8	7	950	750	2,98	2,88	2,93	5000	2800		5000	2400	2400			2400	3,70	3,38	3,54	3	b
			1000	0	1	2	2																			
2553	2006	Infant formula milk powder	100	12	5	12	6	850	900	2,93	2,95	2,94	1100	1200		1100	1000	2500			1000	3,04	3,00	3,02	3	b
			100	2	0	0	1																			
4714	2010	Raw cow milk	100	>150	50	>150	>150	68000	67000	4,83	4,83	4,83	>49000	52000		52000	>49000	40000			40000	4,72	4,60	4,66	3	c
			1000	84	51	60	74																			
4715	2010	Raw cow milk	10	42	41	42	34	420	400	2,62	2,60	2,61	360	330		360	460	450			460	2,56	2,66	2,61	3	c
			100	4	6	5	6																			
4716	2010	Raw cow milk	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100			<10	<1,00	<1,00	<1,00	3	c
			100	0	0	0	0																			
4717	2010	Raw cow milk	10	0	1	1	0	5	5	0,70	0,70	0,70*	<10	<100		<10	<10	<100			<10	<1,00	<1,00	0,70*	3	c
			100	0	0	0	0																			
4912	2010	Raw cow milk	10	7	4	4	8	55	60	1,74	1,78	1,76	45	100		45	69	<100			69	1,65	1,84	1,75	3	c
			100	2	2	0	0																			
4913	2010	Raw cow milk	100	0	0	0	0	<100	<100	<2,00	<2,00	<2,00	21	<100		21	10	<100			10	1,32	1,00	1,16	3	c
			1000	0	0	0	0																			
4914	2010	Raw cow milk	100	0	0	0	0	<100	<100	<2,00	<2,00	<2,00	10	<100		10	<10	<100			<10	1,00	<1,00	1,00	3	c
			1000	0	0	0	0																			
5054	2010	Raw cow milk	10	28	28	31	22	270	270	2,43	2,43	2,43	230	100	<1000	230	210	100	<1000		210	2,36	2,32	2,34	3	c
			100	1	2	3	3																			

DAIRY PRODUCTS																								Category	Type
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC												
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1			Replicate 2			Replicate 1	Replicate 2	Mean				
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g	
5055	2010	Raw cow milk	10	1	0	1	0	5	5	0,70	0,70	0,70*	<10	<100		<10	<10	<100	<10	<1,00	<1,00	0,70*	3	c	
			100	0	1	0	0																		
5056	2010	Raw cow milk	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100	<10	<1,00	<1,00	<1,00	3	c	
			100	0	0	0	0																		
5100	2010	Raw cow milk	10	11	17	11	10	140	95	2,15	1,98	2,06	44	430		44	140	<100	140	1,64	2,15	1,89	3	c	
			100	1	1	0	0																		
5101	2010	Raw cow milk	10	54	61	57	55	580	550	2,76	2,74	2,75	73	4400		73	440	210	440	1,86	2,64	2,25	3	c	
			100	8	4	5	4																		
5102	2010	Raw cow milk	100	18	19	10	20	1900	1500	3,28	3,18	3,23	930	2600		930	1600	2100	1600	2,97	3,20	3,09	3	c	
			1000	3	1	2	2																		
5103	2010	Raw cow milk	100	31	34	43	36	3300	3700	3,52	3,57	3,54	4400	2400		4400	3400	5300	3400	3,64	3,53	3,59	3	c	
			1000	3	5	3	0																		
5104	2010	Raw cow milk	100	27	18	20	31	2200	2600	3,34	3,41	3,38	1400	5200		1400	5500	4400	5500	3,15	3,74	3,44	3	c	
			1000	2	2	3	4																		
5105	2010	Raw cow milk	100	15	17	19	23	1700	2300	3,23	3,36	3,30	2600	2100		2600	1400	2300	1400	3,41	3,15	3,28	3	c	
			1000	4	1	3	6																		
5112	2010	Raw goat milk	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100	<10	<1,00	<1,00	<1,00	3	c	
			100	0	0	0	0																		
5113	2010	Raw goat milk	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100	<10	<1,00	<1,00	<1,00	3	c	
			100	0	0	0	0																		
5114	2010	Raw goat milk	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100	<10	<1,00	<1,00	<1,00	3	c	
			100	0	0	0	0																		
159	2004	Cooked bacon	10	39	36	36	36	370	360	2,57	2,56	2,56	790	210		790	530	450	530	2,90	2,72	2,81	3	c	
			100	3	4	3	4																		

SEAFOOD																									Category	Type
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC													
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1				Replicate 2				Replicate 1	Replicate 2	Mean			
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g			
199	2004	Fish fillet	10	4	2	3	1	30	20	1,48	1,30	1,39	110	<100	<1000	110	170	<100	<1000	170	2,04	2,23	2,14	4	a	
			100	1	0	0	0																			
200	2004	Raw Clams	10	0	1	0	0	5	<10	0,70	<1	<1,00	/	No result (error 19)		/	/	No result (error 19)		/	No result (error 19)	No result (error 19)	No result (error 19)	4	a	
			100	0	0	0	0																			
201	2004	Raw Shells	10	1	1	0	0	10	<10	1,00	<1	<1,00	/	<100		<100	/	100		100	<100	100	<2,00	4	a	
			100	0	0	0	0																			
221	2004	Raw Oysters	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	/	<100		/	/	<100		/	<2,00	<2,00	<2,00	4	a	
			100	0	0	0	0																			
222	2004	Sardines	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	4	a	
			100	0	0	0	0																			
223	2004	Trout	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	4	a	
			100	0	0	0	0																			
256	2004	Raw sea almonds	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	/	<100		/	/	<100		/	<2,00	<2,00	<2,00	4	a	
			100	0	0	0	0																			
257	2004	Raw clams	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	/	<100		/	/	<100		/	<2,00	<2,00	<2,00	4	a	
			100	0	0	0	0																			
258	2004	Raw oysters	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	/	<100		<100	/	<100		<100	<2,00	<2,00	<2,00	4	a	
			100	0	0	0	0																			
278	2004	Cooked mussels	10	130	125	135	150	1300	1400	3,11	3,15	3,13	1300	1500		1300	1000	2600		1000	3,11	3,00	3,06	4	a	
			100	12	15	12	15																			
279	2004	Tuna	10	8	7	8	3	77	59	1,89	1,77	1,83	89	<100		89	150	<100		150	1,95	2,18	2,06	4	a	
			100	0	2	0	2																			
280	2004	Fish fillet	10	25	13	22	23	210	230	2,32	2,36	2,34	300	210		300	230	<100		230	2,48	2,36	2,42	4	a	
			100	4	3	2	4																			
7453	2016	Raw shrimps	100	80				7900		3,90		3,90	12000	11000		12000					4,08		4,08	4	a	
			1000	7																						
246	2004	Ready to reheat fish	10	6	7	7	7	65	70	1,81	1,85	1,83	89	<100		89	57	100		57	1,95	1,76	1,85	4	b	
			100	1	0	0	3																			
247	2004	Ready to reheat fish	10	>150	>150	138	>150	1800	1500	3,26	3,18	3,22	1700	2300		1700	2000	3900		2000	3,23	3,30	3,27	4	b	
			100	20	15	20	27																			
248	2004	Ready to reheat fish	10	11	9	4	9	100	70	2,00	1,85	1,92	99	210		99	130	100		130	2,00	2,11	2,05	4	b	
			100	0	1	1	0																			
7454	2016	Shrimps with spicy sauce	100	87				9300		3,97		3,97	No result (error 19)	16000		16000					4,20		4,20	4	b	
			1000	15																						
7455	2016	Cod fritters	1000	51				53000		4,72		4,72	>49000	91000	73000	91000					4,96		4,96	4	b	
			10000	7																						
290	2004	Smoked fish	10	98	100	87	93	960	860	2,98	2,93	2,96	2000	1900		2000	1600	2300		1600	3,30	3,20	3,25	4	c	
			100	6	7	5	5																			
291	2004	Smoked fish	10	67	55	67	74	610	690	2,79	2,84	2,81	930	890		930	1500	1000		1500	2,97	3,18	3,07	4	c	
			100	5	6	4	7																			
292	2004	Smoked fish	10	97	90	90	86	920	870	2,96	2,94	2,95	830	1600		830	1200	590		1200	2,92	3,08	3,00	4	c	
			100	8	7	8	7																			
7456	2016	Smoked trout	100	71				6800		3,83		3,83	11000	17000		11000					4,04		4,04	4	c	
			1000	4																						
7457	2016	Smoked mackerel	1000	57				54000		4,73		4,73	>49000	110000	95000	110000					5,04		5,04	4	c	
			10000	2																						

FRUITS AND VEGETABLES																								Category	Type
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC												
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean log CFU/g	Replicate 1			Replicate 2			Replicate 1 log CFU/g	Replicate 2 log CFU/g	Mean log CFU/g				
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g		d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result					
198	2004	Broccoli	10	9	2	3	4	60	40	1,78	1,60	1,69	260	100	<1000	260	280	<100	<1000	280	2,41	2,45	2,43	5	a
			100	0	0	0	0																		
219	2004	Beans	10	5	7	4	7	60	60	1,78	1,78	1,78	71	<100		71	59	100		59	1,85	1,77	1,81	5	a
			100	1	0	0	0																		
249	2004	Buckwheat seed	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
259	2004	Carrots	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
260	2004	Turnips	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
261	2004	Green cabbage	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
262	2004	Figs	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
269	2004	Figs	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
282	2004	Cabbage	10	2	0	1	0	10	5	1,00	0,70	0,85*	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	a
			100	0	0	0	0																		
287	2004	Lettuce	10	62	52	56	63	560	570	2,75	2,76	2,75	3400	2800		3400	1900	1900		1900	3,53	3,28	3,41	5	a
			100	0	8	4	2																		
288	2004	Salad	10	162	164	97	136	1600	1100	3,20	3,04	3,12	4100	6200		4100	4400	9500		4400	3,61	3,64	3,63	5	a
			100	10	10	11	5																		
7458	2016	Zucchini	1000	50				52000		4,72		4,72	49000	91000	130000	49000					4,69		4,69	5	a
			10000	7																					
263	2004	Dried pears	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	b
			100	0	0	0	0																		
267	2004	Dried apricots	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	b
			100	0	0	0	0																		
268	2004	Pistachios	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	b
			100	0	0	0	0																		
281	2004	Grated carrots	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	b
			100	0	0	0	0																		
283	2004	Mixed salad	10	3	1	2	1	20	15	1,30	1,18	1,24*	10	<100		10	10	<100		10	1,00	1,00	1,00	5	b
			100	0	0	0	0																		
293	2004	Dried apple	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	210		210	<10	590		590	2,32	2,77	2,55	5	b
			100	0	0	0	0																		
294	2004	Dried apple	10	34	18	33	28	260	310	2,41	2,49	2,45	<10	450		450	450	450		450	2,65	2,65	2,65	5	b
			100	3	2	3	3																		
2121	2011	Ready to eat salad	100	105				10000		4,00		4,00	4500	6400		4500					3,65		3,65	5	b
			1000	7																					
2123	2011	Tabbouleh	10	0				<10		<1,00		<1,00	<10	<100		<10					<1,00		<1,00	5	b
			100	0																					
2363	2011	Salad carrots celery	10	42				430		2,63		2,63	360	330		360					2,56		2,56	5	b
			100	5																					
2364	2011	Celery remoulade	100	20				2300		3,36		3,36	3100	2800		3100					3,49		3,49	5	b
			1000	5																					
2365	2011	Mixed vegetables	10	25				240		2,38		2,38	160	100		160					2,20		2,20	5	b
			100	1																					
2366	2011	Mixed vegetables	100	>150				77000		4,89		4,89		>49000	110000	110000					5,04		5,04	5	b
			1000	77				N'																	
7202	2016	Tabbouleh	1000	16				15000		4,18		4,18	21000	37000		21000					4,32		4,32	5	b
			10000	1																					

\* Analyses performed according to the COFRAC accreditation

FRUITS AND VEGETABLES																								Category	Type
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC												
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1				Replicate 2				Replicate 1	Replicate 2	Mean		
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g		
7203	2016	Grated carrots	1000	19				17000		4,23		4,23	37000	34000		37000					4,57		4,57	5	b
			10000	0																					
7204	2016	Cucumbers with cream	1000	58			61000		4,79		4,79	>49000	210000	64000	210000						5,32		5,32	5	b
			10000	9																					
196	2004	Ready to reheat vegetables	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100	<1000	<10	<10	<100	<1000	<10	<1,00	<1,00	<1,00	5	c
			100	0	0	0	0																		
197	2004	Ready to reheat vegetables	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100	<1000	<10	<10	<100	<1000	<10	<1,00	<1,00	<1,00	5	c
			100	0	0	0	0																		
220	2004	Ready to reheat vegetables	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	c
			100	0	0	0	0																		
245	2004	Ready to reheat food	10	36	29	44	37	330	400	2,52	2,60	2,56	430	210		430	300	730		300	2,63	2,48	2,56	5	c
			100	4	3	3	3																		
253	2004	Frozen ready to reheat vegetables	100	67	67	55	62	6500	6100	3,81	3,79	3,80	15000	12000		15000	15000	22000		15000	4,18	4,18	4,18	5	c
			1000	4	5	11	6																		
254	2004	Frozen ready to reheat vegetables	10	19	22	34	25	200	270	2,30	2,43	2,37	420	<100		420	330	330		330	2,62	2,52	2,57	5	c
			100	1	2	0	1																		
255	2004	Frozen ready to reheat vegetables	10	30	61	28	40	440	330	2,64	2,52	2,58	330	320		330	530	330		530	2,52	2,72	2,62	5	c
			100	2	4	2	2																		
270	2004	Dried pears	10	0	0	0	0	<10	<10	<1,00	<1,00	<1,00	<10	<100		<10	<10	<100		<10	<1,00	<1,00	<1,00	5	c
			100	0	0	0	0																		
289	2004	Dried mushrooms	10	23	30	28	27	280	290	2,45	2,46	2,45	810	1100		810	930	320		930	2,91	2,97	2,94	5	c
			100	5	4	3	6																		
2058	2011	Ratatouille	10	11				120		2,08		2,08	68	<100		68					1,83		1,83	5	c
			100	2																					
2060	2011	Ready to cook vegetables	100	29				2900		3,46		3,46	2000	1900		2000					3,30		3,30	5	c
			1000	3																					
2120	2011	Frozen ratatouille	10	51				510		2,71		2,71	300	710		300					2,48		2,48	5	c
			100	5																					

PET FOODS																								Category	Type
N° Sample	Analysis date	Product	Dilution	Reference method: NF ISO 16649-2*									Alternative method: TEMPO EC												
				Rep1		Rep2		Rep1	Rep2	Replicate 1	Replicate 2	Mean	Replicate 1				Replicate 2				Replicate 1	Replicate 2	Mean		
				Plate a	Plate b	Plate a	Plate b	CFU/g	CFU/g	log CFU/g	log CFU/g	log CFU/g	d 1/40	d 1/400	d 1/4000	Result	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	log CFU/g	log CFU/g		
226	2004	Raw meat for animals	10	2	4	7	6	30	65	1,48	1,81	1,65	380	320		380	500	440		500	2,58	2,70	2,64	6	a
			100	0	0	1	0																		
227	2004	Raw meat for animals	10	19	31	25	30	240	260	2,38	2,41	2,40	790	450		790	530	570		530	2,90	2,72	2,81	6	a
			100	3	0	1	1																		
228	2004	Raw meat for animals	10	9	3	6	4	60	50	1,78	1,70	1,74	PDR19	210		210	PDR19	100		100	2,32	2,00	2,16	6	a
			100	0	0	0	1																		
7459	2016	Raw meat for animals	100	112				11000		4,04		4,04	21000	12000		21000					4,32		4,32	6	a
			1000	13																					
7460	2016	Raw meat for animals	1000	101				110000		5,04		5,04	>49000	300000	140000	300000					5,48		5,48	6	a
			10000	10																					
271	2004	Sausage for dog	10	115	110	220	130	1200	1300	3,08	3,11	3,10	7800	4000		7800	4400	4200		4400	3,89	3,64	3,77	6	b
			100	10	19	20	20																		
272	2004	Sausage for dog	10	31	20	16	22	240	180	2,38	2,26	2,32	1700	1800		1700	2600	2200		2600	3,23	3,41	3,32	6	b
			100	1	1	0	2																		
273	2004	Pâté for dog	100	62	29	44	45	4300	4200	3,63	3,62	3,63	30000	34000		30000	37000	17000		37000	4,48	4,57	4,52	6	b
			1000	2	2	3	1																		
274	2004	Pâté for dog	100	81	84	100	76	8000	8500	3,90	3,93	3,92	>49000	91000		91000	>49000	60000		60000	4,96	4,78	4,87	6	b
			1000	7	4	8	4																		
7205	2016	Sausage for dog	10	10				100		2,00		2,00	<10	<100		<10					<1,00		<1,00	6	b
			100	1																					
7461	2016	Pâté for dog	100	>150				24000 N'		4,38 N'		4,38 N'	15000	15000		15000					4,18		4,18	6	b
			1000	24																					
250	2004	Pellets for kitten	10	19	12	12	8	160	100	2,20	2,00	2,10	140	330		140	250	210		250	2,15	2,40	2,27	6	c
			100	1	3	0	2																		
251	2004	Pellets	100	55	54	35	63	5300	4900	3,72	3,69	3,71	4500	6400		4500	5000	7100		5000	3,65	3,70	3,68	6	c
			1000	4	3	4	6																		
252	2004	Pellets	10	81	88	66	91	840	820	2,92	2,91	2,92	4100	2000		4100	2300	2300		2300	3,61	3,36	3,49	6	c
			100	7	9	17	6																		
7206	2016	Pellets for cats	10	10				91		1,96		1,96	<10	<100		<10					<1,00		<1,00	6	c
			100	0																					
7207	2016	Pellets for cats	10	53				560		2,75		2,75	32	210		32					1,51		1,51	6	c
			100	9																					
7462	2016	Pellets for dog	1000	>150				290000 N'		5,46 N'		5,46 N'	>49000	91000	120000	91000					4,96		4,96	6	c
			10000	29																					



### Appendix 5 - Relative trueness study: statistical calculations

Category	Type	N° sample	Incubation: 24 - 27 h at 37°C ±1 °C									
			Log cfu/g		Mean	Diffe- rence	Alternative method		Mean <4 CFU/ plate	Diffe- rence <4 CFU/ plate	Mean corrected values	Difference Corrected values
			Reference method	Alternative method			<4 CFU/ plate	<or> threshold corrected values				
1	a	2062	2,34	2,20	2,27	-0,14			#N/A		#N/A	
	a	2063	3,63	3,49	3,56	-0,14			#N/A		#N/A	
	a	2064	4,69	4,60	4,65	-0,09			#N/A		#N/A	
	a	2122	0,00		#N/A		0,00		#N/A		0,00	0,00
	a	2150	2,38	2,15	2,26	-0,23			#N/A		#N/A	
	a	2151	3,71	3,49	3,60	-0,22			#N/A		#N/A	
	a	2184	3,67	3,53	3,60	-0,14			#N/A		#N/A	
	a	2368	3,65	3,28	3,47	-0,37			#N/A		#N/A	
	a	2480	2,28	2,34	2,31	0,06			#N/A		#N/A	
	a	2481	2,20	2,20	2,20	0,00			#N/A		#N/A	
	b	160	0,00		#N/A		0,00		#N/A		0,00	0,00
	b	244	3,04	3,78	3,41	0,74			#N/A		#N/A	
	b	277	3,63	4,32	3,98	0,69			#N/A		#N/A	
	b	2059	3,28	3,32	3,30	0,04			#N/A		#N/A	
	b	2061	0,00		#N/A		0,00		#N/A		0,00	0,00
	b	2065	3,85	3,78	3,81	-0,07			#N/A		#N/A	
	b	2066	3,04	2,41	2,73	-0,63			#N/A		#N/A	
	b	2183	4,81	4,78	4,80	-0,03			#N/A		#N/A	
	b	2482	2,97	2,66	2,82	-0,31			#N/A		#N/A	
	c	159	2,57	2,90	2,73	0,33			#N/A		#N/A	
c	2369	3,26	2,38	2,82	-0,88			#N/A		#N/A		
c	2470	2,91	2,76	2,83	-0,16			#N/A		#N/A		
c	2472	3,57	3,46	3,52	-0,11			#N/A		#N/A		
c	2473	3,83	3,83	3,83	0,01			#N/A		#N/A		
Average category 1							-0,08					
Standard deviation of differences category 1							0,36					
2	a	161	4,41	4,76	4,59	0,35			#N/A		#N/A	
	a	162	1,91	2,04	1,98	0,13			#N/A		#N/A	
	a	275	2,79	3,05	2,92	0,26			#N/A		#N/A	
	a	1911	3,04	2,91	2,97	-0,13			#N/A		#N/A	
	a	1912	2,96	2,98	2,97	0,02			#N/A		#N/A	
	a	1918	4,00	3,96	3,98	-0,04			#N/A		#N/A	
	a	1919	4,72	4,64	4,68	-0,08			#N/A		#N/A	
	b	224	3,16	3,34	3,25	0,17			#N/A		#N/A	
	b	225	2,87	3,18	3,02	0,30			#N/A		#N/A	
	b	276	3,36	3,77	3,56	0,41			#N/A		#N/A	
	b	1866	2,75	2,95	2,85	0,21			#N/A		#N/A	
	b	1867	2,71	2,34	2,52	-0,37			#N/A		#N/A	
	b	1868	3,77	3,78	3,77	0,01			#N/A		#N/A	
	b	1913	3,73	3,89	3,81	0,16			#N/A		#N/A	
	b	1915	4,23	4,32	4,28	0,09			#N/A		#N/A	
	b	1916	3,15	2,97	3,06	-0,18			#N/A		#N/A	
	b	1917	1,74	2,45	2,09	0,71			#N/A		#N/A	
	b	2186	2,20	2,20	2,20	0,00			#N/A		#N/A	
	c	1865	1,00		#N/A		0,00		#N/A		0,50	-1,00
	c	1869	3,00	2,91	2,95	-0,09			#N/A		#N/A	
c	1870	2,90	2,86	2,88	-0,04			#N/A		#N/A		
c	1871	2,69	2,63	2,66	-0,06			#N/A		#N/A		
c	2152	1,60	1,52	1,56	-0,08			#N/A		#N/A		
c	2153	3,62	3,53	3,58	-0,09			#N/A		#N/A		
c	2185	2,20	1,65	1,93	-0,55			#N/A		#N/A		
c	2475	1,85	1,77	1,81	-0,07			#N/A		#N/A		
c	2476	1,70	1,95	1,82	0,25			#N/A		#N/A		
c	2478	4,32	4,23	4,28	-0,09			#N/A		#N/A		
c	2479	4,79	4,96	4,88	0,17			#N/A		#N/A		
Average category 2							0,05					
Standard deviation of differences category 2							0,25					

Category	Type	N° sample	Incubation: 24 - 27 h at 37°C ±1 °C									
			Log cfu/g		Mean	Difference	Alternative method		Mean <4 CFU/ plate	Difference <4 CFU/ plate	Mean corrected values	Difference Corrected values
			Reference method	Alternative method			<4 CFU/ plate	<or> threshold corrected values				
3	a	193	1,39		#N/A		1,88		1,64	0,49	#N/A	
	a	194	2,11	3,50	2,80	1,38			#N/A		#N/A	
	a	195	1,69	2,30	1,99	0,61			#N/A		#N/A	
	a	240	2,93	2,83	2,88	-0,10			#N/A		#N/A	
	a	241	0,00		#N/A			2,11	#N/A		1,06	2,11
	a	242	2,97	2,75	2,86	-0,21			#N/A		#N/A	
	a	243	3,10	3,09	3,10	0,00			#N/A		#N/A	
	a	264	4,31	4,52	4,42	0,21			#N/A		#N/A	
	b	2504	1,05		#N/A		1,00		1,02	-0,05	#N/A	
	b	2505	2,26	1,64	1,95	-0,62			#N/A		#N/A	
	b	2506	2,97	2,95	2,96	-0,01			#N/A		#N/A	
	b	2507	4,06	4,02	4,04	-0,04			#N/A		#N/A	
	b	2508	3,99	4,00	3,99	0,01			#N/A		#N/A	
	b	2509	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	2510	0,85		#N/A			0,00	#N/A		0,42	-0,85
	b	2511	2,39		#N/A			0,00	#N/A		1,19	-2,39
	b	2513	3,55	3,39	3,47	-0,16			#N/A		#N/A	
	b	2514	1,63	1,93	1,78	0,30			#N/A		#N/A	
	b	2515	2,22	2,24	2,23	0,02			#N/A		#N/A	
	b	2516	2,86	2,89	2,87	0,03			#N/A		#N/A	
	b	2517	3,16	3,25	3,21	0,09			#N/A		#N/A	
	b	2518	3,15	3,53	3,34	0,38			#N/A		#N/A	
	b	2519	3,37	3,57	3,47	0,20			#N/A		#N/A	
	b	2520	4,40	4,44	4,42	0,05			#N/A		#N/A	
	b	2521	4,35	4,58	4,47	0,23			#N/A		#N/A	
	b	2522	4,60	4,74	4,67	0,14			#N/A		#N/A	
	b	2523	5,01	5,02	5,02	0,00			#N/A		#N/A	
	b	2524	3,69	3,90	3,79	0,21			#N/A		#N/A	
	b	2525	3,92	3,62	3,77	-0,29			#N/A		#N/A	
	b	2546	1,39		#N/A		1,00		1,19	-0,39	#N/A	
	b	2547	1,53		#N/A		1,16		1,34	-0,37	#N/A	
	b	2548	1,95	2,00	1,98	0,05			#N/A		#N/A	
	b	2549	2,02	2,04	2,03	0,02			#N/A		#N/A	
	b	2550	2,31	2,60	2,46	0,29			#N/A		#N/A	
	b	2551	2,20	2,53	2,37	0,32			#N/A		#N/A	
	b	2552	2,93	3,54	3,23	0,61			#N/A		#N/A	
	b	2553	2,94	3,02	2,98	0,08			#N/A		#N/A	
	c	4714	4,83	4,66	4,74	-0,17			#N/A		#N/A	
	c	4715	2,61	2,61	2,61	0,00			#N/A		#N/A	
	c	4716	0,00		#N/A			0,00	#N/A		0,00	0,00
c	4717	0,70		#N/A		0,70		0,70	0,00	#N/A		
c	4912	1,76	1,75	1,75	-0,01			#N/A		#N/A		
c	4913	1,00		#N/A			1,16	#N/A		1,08	0,16	
c	4914	1,00		#N/A			1,00	#N/A		1,00	0,00	
c	5054	2,43	2,34	2,39	-0,09			#N/A		#N/A		
c	5055	0,70		#N/A		0,70		0,70	0,00	#N/A		
c	5056	0,00		#N/A			0,00	#N/A		0,00	0,00	
c	5100	2,06	1,89	1,98	-0,17			#N/A		#N/A		
c	5101	2,75	2,25	2,50	-0,50			#N/A		#N/A		
c	5102	3,23	3,09	3,16	-0,14			#N/A		#N/A		
c	5103	3,54	3,59	3,57	0,04			#N/A		#N/A		
c	5104	3,38	3,44	3,41	0,06			#N/A		#N/A		
c	5105	3,30	3,28	3,29	-0,02			#N/A		#N/A		
c	5112	0,00		#N/A			0,00	#N/A		0,00	0,00	
c	5113	0,00		#N/A			0,00	#N/A		0,00	0,00	
c	5114	0,00		#N/A			0,00	#N/A		0,00	0,00	
Average category 3												
Standard deviation of differences category 3							0,07					
							0,32					



Category	Type	N° sample	Incubation : 24 - 27 h at 37°C ±1 °C									
			Log cfu/g		Mean	Difference	Alternative method		Mean <4 CFU/ plate	Difference <4 CFU/ plate	Mean corrected values	Difference Corrected values
			Reference method	Alternative method			<4 CFU/ plate	<or> threshold corrected values				
4	a	199	1,39	2,14	1,76	0,75			#N/A		#N/A	
	a	201	0,00		#N/A			1,00	#N/A		0,50	1,00
	a	221	0,00		#N/A			1,00	#N/A		0,50	1,00
	a	222	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	223	0,00		#N/A			1,00	#N/A		0,50	1,00
	a	256	0,00		#N/A			1,00	#N/A		0,50	1,00
	a	257	0,00		#N/A			1,00	#N/A		0,50	1,00
	a	278	3,13	3,06	3,09	-0,07			#N/A		#N/A	
	a	279	1,83	2,06	1,95	0,23			#N/A		#N/A	
	a	280	2,34	2,42	2,38	0,08			#N/A		#N/A	
	a	7453	3,90	4,08	3,99	0,18			#N/A		#N/A	
	b	246	1,83	1,85	1,84	0,02			#N/A		#N/A	
	b	247	3,22	3,27	3,24	0,05			#N/A		#N/A	
	b	248	1,92	2,05	1,99	0,13			#N/A		#N/A	
	b	7454	3,97	4,20	4,09	0,24			#N/A		#N/A	
	b	7455	4,72	4,96	4,84	0,23			#N/A		#N/A	
	c	290	2,96	3,25	3,11	0,29			#N/A		#N/A	
	c	291	2,81	3,07	2,94	0,26			#N/A		#N/A	
	c	292	2,95	3,00	2,98	0,05			#N/A		#N/A	
	c	7456	3,83	4,04	3,94	0,21			#N/A		#N/A	
c	7457	4,73	5,04	4,89	0,31			#N/A		#N/A		
Average category 4							0,20					
Standard deviation of differences category 4							0,19					
5	a	198	1,69	2,43	2,06	0,74			#N/A		#N/A	
	a	219	1,78	1,81	1,79	0,03			#N/A		#N/A	
	a	249	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	259	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	260	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	261	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	262	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	269	0,00		#N/A			0,00	#N/A		0,00	0,00
	a	282	0,85		#N/A			0,00	#N/A		0,42	-0,85
	a	287	2,75	3,41	3,08	0,65			#N/A		#N/A	
	a	288	3,12	3,63	3,38	0,51			#N/A		#N/A	
	a	7458	4,72	4,69	4,70	-0,03			#N/A		#N/A	
	b	256	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	263	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	267	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	268	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	281	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	283	1,24	1,00	1,12	-0,24			#N/A		#N/A	
	b	293	0,00		#N/A			2,55	#N/A		1,27	2,55
	b	294	2,45	2,65	2,55	0,20			#N/A		#N/A	
	b	2121	4,00	3,65	3,83	-0,35			#N/A		#N/A	
	b	2123	0,00		#N/A			0,00	#N/A		0,00	0,00
	b	2363	2,63	2,56	2,59	-0,08			#N/A		#N/A	
	b	2364	3,36	3,49	3,43	0,13			#N/A		#N/A	
	b	2365	2,38	2,20	2,29	-0,18			#N/A		#N/A	
	b	2366	4,89	5,04	4,96	0,15			#N/A		#N/A	
	b	7202	4,18	4,32	4,25	0,15			#N/A		#N/A	
	b	7203	4,23	4,57	4,40	0,34			#N/A		#N/A	
	b	7204	4,79	5,32	5,05	0,54			#N/A		#N/A	
	c	196	0,00		#N/A			0,00	#N/A		0,00	0,00
	c	197	0,00		#N/A			0,00	#N/A		0,00	0,00
	c	220	0,00		#N/A			0,00	#N/A		0,00	0,00
	c	245	2,56	2,56	2,56	0,00			#N/A		#N/A	
c	253	3,80	4,18	3,99	0,38			#N/A		#N/A		
c	254	2,37	2,57	2,47	0,20			#N/A		#N/A		
c	255	2,58	2,62	2,60	0,04			#N/A		#N/A		
c	270	0,00		#N/A			0,00	#N/A		0,00	0,00	
c	289	2,45	2,94	2,70	0,48			#N/A		#N/A		
c	2058	2,08	1,83	1,96	-0,25			#N/A		#N/A		
c	2060	3,46	3,30	3,38	-0,16			#N/A		#N/A		
c	2120	2,71	2,48	2,59	-0,23			#N/A		#N/A		
Average category 5							0,13					
Standard deviation of differences category 5							0,31					



### Appendix 6 - Accuracy profile study: raw data

Matrix	Strain	Level	Sample N°	Reference method: ISO 16649-2♦				Alternative method : TEMPO EC				
				Dilution	cfu/plate	cfu/g	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Liver pâté Batch 1 Aerobic mesophilic flora : 4.0.10 <sup>6</sup> CFU/g	Escherichia coli 9	1	7504	10	27	300	2,48	400	440	400	2,60	
				100	6							
			7505	10	39	360	2,56	270	450	270	2,43	
				100	1							
			7506	10	29	310	2,49	310	210	310	2,49	
				100	5							
		7507	10	29	270	2,43	330	330	330	2,52		
			100	1								
		7508	10	23	230	2,36	360	210	360	2,56		
			100	2								
		2	7509	100	50	5100	3,71	12000	8100	12000	4,08	
				1000	6							
			7510	100	48	4800	3,68	11000	8300	11000	4,04	
				1000	5							
			7511	100	62	6400	3,81	9100	5800	9100	3,96	
				1000	8							
		7512	100	52	5500	3,74	21000	6300	21000	4,32		
			1000	9								
		3	7513	100	47	4700	3,67	11000	7100	11000	4,04	
				1000	5							
			7514	1000	90	96000	4,98		490000	140000	490000	5,69
				10000	16							
			7515	1000	93	95000	4,98		250000	170000	250000	5,40
				10000	12							
7516	1000	93	100000	5,00		300000	120000	300000	5,48			
	10000	19										
7517	1000	91	94000	4,97		150000	180000	150000	5,18			
	10000	12										
7518	1000	100	99000	5,00		150000	190000	150000	5,18			
	10000	9										
Liver pâté Batch 2 Aerobic mesophilic flora : 2.8.10 <sup>5</sup> CFU/g	Escherichia coli 9	1	7519	10	22	210	2,32	300	210	300	2,48	
				100	1							
			7520	10	23	220	2,34	360	210	360	2,56	
				100	1							
			7521	10	30	290	2,46	300	330	300	2,48	
				100	2							
		7522	10	23	220	2,34	400	1400	400	2,60		
			100	1								
		7523	10	26	260	2,41	280	590	280	2,45		
			100	2								
		2	7524	100	58	5900	3,77	7800	8300	7800	3,89	
				1000	7							
			7525	100	52	4900	3,69	12000	5700	12000	4,08	
				1000	2							
			7526	100	58	6200	3,79	12000	6300	12000	4,08	
				1000	10							
		7527	100	50	4800	3,68	7800	14000	7800	3,89		
			1000	3								
		3	7528	100	58	5900	3,77	7800	12000	7800	3,89	
				1000	7							
			7529	1000	85	84000	4,92		170000	160000	170000	5,23
				10000	7							
			7530	1000	84	87000	4,94		210000	440000	210000	5,32
				10000	12							
7531	1000	85	87000	4,94		150000	110000	150000	5,18			
	10000	11										
7532	1000	92	99000	5,00		250000	110000	250000	5,40			
	10000	17										
7533	1000	96	95000	4,98		210000	140000	210000	5,32			
	10000	8										

♦ Analyses performed according to the COFRAC accreditation

Matrix	Strain	Level	Sample N°	Reference method : ISO 16649-2*				Alternative method : TEMPO EC				
				Dilution	cfu/plate	cfu/g	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Ground beef Batch 1 Aerobic mesophilic flora : 1, 1.10 <sup>4</sup> CFU/g	Escherichia coli 13	1	7650	10	37	360	2,56	360	320		360	2,56
				100	2							
			7651	10	32	340	2,53	620	330		620	2,79
				100	5							
			7652	10	34	360	2,56	440	440		440	2,64
				100	6							
		7653	10	41	390	2,59	730	210		730	2,86	
			100	2								
		7654	10	36	350	2,54	640	320		640	2,81	
			100	2								
		2	7655	100	65	6200	3,79	6000	10000		6000	3,78
				1000	3							
			7656	100	79	7600	3,88	7800	8300		7800	3,89
				1000	5							
			7657	100	61	5900	3,77	5000	8300		5000	3,70
				1000	4							
		7658	100	55	5500	3,74	5500	9500		5500	3,74	
			1000	6								
		7659	100	56	5800	3,76	5500	10000		5500	3,74	
			1000	8								
		3	7660	1000	94	95000	4,98		210000	140000	210000	5,32
				10000	11							
			7661	1000	86	88000	4,94		300000	190000	300000	5,48
				10000	11							
7662	1000		93	98000	4,99		210000	140000	210000	5,32		
	10000		15									
7663	1000	115	110000	5,04		170000	220000	170000	5,23			
	10000	9										
7664	1000	88	80000	4,90		210000	180000	210000	5,32			
	10000	0										
Ground beef Batch 2 Aerobic mesophilic flora : 2, 7.10 <sup>3</sup> CFU/g	Escherichia coli 13	1	7665	10	47	470	2,67	710	730		710	2,85
				100	5							
			7666	10	25	270	2,43	250	210		250	2,40
				100	5							
			7667	10	34	340	2,53	570	860		570	2,76
				100	3							
		7668	10	35	350	2,54	330	330		330	2,52	
			100	3								
		7669	10	35	370	2,57	580	210		580	2,76	
			100	6								
		2	7670	100	66	6400	3,81	6000	10000		6000	3,78
				1000	4							
			7671	100	56	5500	3,74	11000	8800		11000	4,04
				1000	5							
			7672	100	56	5800	3,76	7400	7300		7400	3,87
				1000	8							
		7673	100	56	5500	3,74	7800	7300		7800	3,89	
			1000	5								
		7674	100	58	5900	3,77	15000	8300		15000	4,18	
			1000	7								
		3	7675	1000	118	120000	5,08		170000	190000	170000	5,23
				10000	16							
			7676	1000	100	110000	5,04		210000	210000	210000	5,32
				10000	17							
7677	1000		125	130000	5,11		250000	200000	250000	5,40		
	10000		23									
7678	1000	100	110000	5,04		300000	310000	300000	5,48			
	10000	16										
7679	1000	86	93000	4,97		370000	180000	370000	5,57			
	10000	16										

\* Analyses performed according to the COFRAC accreditation

Matrix	Strain	Level	Sample N°	Reference method : ISO 16649-2*				Alternative method : TEMPO EC				
				Dilution	cfu/plate	cfu/g	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Pasteurized milk Batch 1 Aerobic mesophilic flora : 4000 CFU/ml	Escherichia coli 94	1	7750	10	18	190	2,28	360	450	360	2,56	
				100	3							
			7751	10	29	320	2,51	240	450	240	2,38	
				100	6							
			7752	10	26	240	2,38	400	440	400	2,60	
				100	0							
		7753	10	22	210	2,32	300	100	300	2,48		
			100	1								
		7754	10	16	170	2,23	260	100	260	2,41		
			100	3								
		2	7755	100	37	3600	3,56	6800	6400	6800	3,83	
				1000	3							
			7756	100	36	3800	3,58	11000	6400	11000	4,04	
				1000	6							
			7757	100	43	4500	3,65	6800	5700	6800	3,83	
				1000	6							
		7758	100	43	4400	3,64	6800	5300	6800	3,83		
			1000	5								
		7759	100	38	3700	3,57	3700	8100	3700	3,57		
			1000	3								
		3	7760	1000	56	55000	4,74		210000	110000	210000	5,32
				10000	5							
			7761	1000	67	67000	4,83		170000	120000	170000	5,23
				10000	7							
7762	1000		68	68000	4,83		120000	310000	120000	5,08		
	10000		7									
7763	1000	72	75000	4,88		210000	240000	210000	5,32			
	10000	10										
7764	1000	65	67000	4,83		250000	140000	250000	5,40			
	10000	9										
Pasteurized milk Batch 2 Aerobic mesophilic flora : <200 CFU/ml	Escherichia coli 94	1	7765	10	34	320	2,51	300	210	300	2,48	
				100	1							
			7766	10	30	330	2,52	240	330	240	2,38	
				100	6							
			7767	10	33	330	2,52	390	590	390	2,59	
				100	3							
		7768	10	35	340	2,53	640	440	640	2,81		
			100	2								
		7769	10	31	330	2,52	360	210	360	2,56		
			100	5								
		2	7770	100	51	4900	3,69	9100	6900	9100	3,96	
				1000	3							
			7771	100	52	5100	3,71	7800	11000	7800	3,89	
				1000	4							
			7772	100	51	5000	3,70	5500	5800	5500	3,74	
				1000	4							
		7773	100	48	5000	3,70	6000	6400	6000	3,78		
			1000	7								
		7774	100	58	6000	3,78	7800	5100	7800	3,89		
			1000	8								
		3	7775	1000	94	97000	4,99		170000	410000	170000	5,23
				10000	13							
			7776	1000	106	110000	5,04		250000	220000	250000	5,40
				10000	13							
7777	1000		98	100000	5,00		170000	170000	170000	5,23		
	10000		12									
7778	1000	85	93000	4,97		170000	270000	170000	5,23			
	10000	17										
7779	1000	93	94000	4,97		250000	310000	250000	5,40			
	10000	10										

\* Analyses performed according to the COFRAC accreditation

Matrix	Strain	Level	Sample N°	Reference method : ISO 16649-2*				Alternative method : TEMPO EC				
				Dilution	cfu/plate	cfu/g	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Grated carrots Batch 1 Aerobic mesophilic flora : >3, 0.10 <sup>5</sup> CFU/g	Escherichia coli 19	1	8239	10	25	260	2,41	400	440		400	2,60
				100	3							
			8240	10	25	240	2,38	280	210		280	2,45
				100	1							
			8241	10	15	150	2,18	360	330		360	2,56
				100	1							
		8242	10	21	200	2,30	230	210		230	2,36	
			100	1								
		8243	10	21	220	2,34	360	100		360	2,56	
			100	3								
		8244	100	25	2500	3,40	4000	7500		4000	3,60	
			1000	3								
		8245	100	29	2700	3,43	7800	6900		7800	3,89	
			1000	1								
		8246	100	38	3700	3,57	3400	4700		3400	3,53	
			1000	3								
		8247	100	36	3800	3,58	5500	3600		5500	3,74	
			1000	6								
		8248	100	29	2800	3,45	5500	7000		5500	3,74	
			1000	2								
		8249	1000	46	48000	4,68		78000	81000	78000	4,89	
			10000	7								
		8250	1000	59	63000	4,80		210000	130000	210000	5,32	
			10000	10								
		8251	1000	60	58000	4,76		68000	83000	68000	4,83	
			10000	4								
		8252	1000	46	45000	4,65		170000	200000	170000	5,23	
10000	4											
8253	1000	48	49000	4,69		91000	81000	91000	4,96			
	10000	6										
Grated carrots Batch 2 Aerobic mesophilic flora : >3, 0.10 <sup>5</sup> CFU(g)	Escherichia coli 19	1	8254	10	13	160	2,20	200	320		200	2,30
				100	5							
			8255	10	31	290	2,46	330	100		330	2,52
				100	1							
			8256	10	19	180	2,26	220	210		220	2,34
				100	1							
		8257	10	18	200	2,30	160	100		160	2,20	
			100	4								
		8258	10	25	250	2,40	400	<100		400	2,60	
			100	2								
		8259	100	24	2500	3,40	5500	8100		5500	3,74	
			1000	4								
		8260	100	22	2600	3,41	6000	4800		6000	3,78	
			1000	7								
		8261	100	37	3500	3,54	5000	5300		5000	3,70	
			1000	2								
		8262	100	31	3200	3,51	3400	5600		3400	3,53	
			1000	4								
		8263	100	33	3300	3,52	4000	8200		4000	3,60	
			1000	3								
		8264	1000	48	44000	4,64		120000	130000	120000	5,08	
			10000	0								
		8265	1000	49	48000	4,68		91000	81000	91000	4,96	
			10000	4								
		8266	1000	45	45000	4,65		150000	120000	150000	5,18	
			10000	5								
		8267	1000	43	45000	4,65		110000	100000	110000	5,04	
10000	7											
8268	1000	44	44000	4,64		210000	210000	210000	5,32			
	10000	4										

\* Analyses performed according to the COFRAC accreditation

Matrix	Strain	Level	Sample N°	Reference method : ISO 16649-2*				Alternative method : TEMPO EC				
				Dilution	cfu/plate	cfu/g	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Frozen fish fillet Batch 1 Aerobic mesophilic flora : 5,2.10 <sup>3</sup> CFU/g	Escherichia coli Ad228	1	8164	10	37	340	2,53	460	330		460	2,66
				100	0							
			8165	10	32	290	2,46	400	330		400	2,60
				100	0							
			8166	10	25	260	2,41	240	450		240	2,38
				100	3							
		8167	10	24	240	2,38	280	440		280	2,45	
			100	2								
		8168	10	20	190	2,28	300	450		300	2,48	
			100	1								
		2	8169	100	52	4900	3,69	7800	4600		7800	3,89
				1000	2							
			8170	100	47	4600	3,66	5300	7000		5300	3,72
				1000	4							
			8171	100	38	4300	3,63	7800	5400		7800	3,89
				1000	9							
		8172	100	43	4200	3,62	6000	7300		6000	3,78	
			1000	3								
		8173	100	40	4200	3,62	4500	8300		4500	3,65	
			1000	6								
		3	8174	1000	58	58000	4,76		110000	110000	110000	5,04
				10000	6							
			8175	1000	57	56000	4,75		170000	120000	170000	5,23
				10000	5							
8176	1000		62	65000	4,81		150000	95000	150000	5,18		
	10000		10									
8177	1000	59	59000	4,77		170000	64000	170000	5,23			
	10000	6										
8178	1000	56	55000	4,74		250000	63000	250000	5,40			
	10000	4										
Frozen fish fillet Batch 2 Aerobic mesophilic flora : 2,5.10 <sup>3</sup> CFU/g	Escherichia coli Ad228	1	8179	10	35	370	2,57	530	890		530	2,72
				100	6							
			8180	10	27	280	2,45	360	590		360	2,56
				100	4							
			8181	10	29	260	2,41	310	100		310	2,49
				100	0							
		8182	10	27	250	2,40	480	330		480	2,68	
			100	0								
		8183	10	46	430	2,63	420	570		420	2,62	
			100	1								
		2	8184	100	50	4700	3,67	4000	1600		4000	3,60
				1000	2							
			8185	100	54	5500	3,74	7800	4300		7800	3,89
				1000	6							
			8186	100	45	4500	3,65	4100	3000		4100	3,61
				1000	5							
		8187	100	45	4500	3,65	5500	3600		5500	3,74	
			1000	4								
		8188	100	37	4100	3,61	4400	3900		4400	3,64	
			1000	8								
		3	8189	1000	79	78000	4,89		78000	150000	78000	4,89
				10000	7							
			8190	1000	79	85000	4,93		120000	150000	120000	5,08
				10000	14							
8191	1000		70	74000	4,87		110000	120000	110000	5,04		
	10000		11									
8192	1000	70	70000	4,85		150000	120000	150000	5,18			
	10000	7										
8193	1000	66	68000	4,83		210000	120000	210000	5,32			
	10000	9										

\* Analyses performed according to the COFRAC accreditation

Matrix	Strain	Level	Sample N°	Reference method : ISO 16649-2*				Alternative method : TEMPO EC				
				Dilution	cfu/plate	cfu/g	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Dog pâté Batch 1 Aerobic mesophilic flora : 10 CFU/g	Escherichia coli 123	1	8269	10	73	760	2,88	1100	860		1100	3,04
				100	11							
			8270	10	83	810	2,91	1400	1600		1400	3,15
				100	6							
			8271	10	69	670	2,83	950	1200		950	2,98
		100		5								
		8272	10	83	800	2,90	1600	710		1600	3,20	
			100	5								
		8273	10	79	760	2,88	570	830		570	2,76	
			100	5								
		2	8274	100	130	13000	4,11	25000	18000		25000	4,40
				1000	13							
			8275	100	141	14000	4,15	30000	19000		30000	4,48
				1000	10							
			8276	100	143	14000	4,15	17000	12000		17000	4,23
		1000		12								
		8277	100	121	12000	4,08	21000	18000		21000	4,32	
			1000	15								
		8278	100	120	11000	4,04	21000	37000		21000	4,32	
			1000	6								
		3	8279	10000	17	200000	5,30		490000	390000	390000	5,59
100000	5											
8280	10000		30	300000	5,48		>490000	290000	290000	5,46		
	100000		3									
8281	10000		28	260000	5,41		3700000	340000	340000	5,53		
	100000	1										
8282	10000	27	290000	5,46		>490000	260000	260000	5,41			
	100000	5										
8283	10000	26	250000	5,40		370000	450000	450000	5,65			
	100000	1										
Dog pâté Batch 2 Aerobic mesophilic flora : 30 CFU/g	Escherichia coli 123	1	8284	10	83	790	2,90	1000	590		1000	3,00
				100	4							
			8285	10	88	870	2,94	1200	1400		1200	3,08
				100	8							
			8286	10	71	740	2,87	1100	1300		1100	3,04
		100		10								
		8287	10	97	940	2,97	1400	590		1400	3,15	
			100	6								
		8288	10	80	800	2,90	1500	710		1500	3,18	
			100	8								
		2	8289	100	130	13000	4,11	25000	4900		25000	4,40
				1000	9							
			8290	100	139	14000	4,15	30000	6400		30000	4,48
				1000	17							
			8291	100	118	12000	4,08	15000	17000		15000	4,18
		1000		15								
		8292	100	118	12000	4,08	21000	13000		21000	4,32	
			1000	9								
		8293	100	152	15000	4,18	17000	14000		17000	4,23	
			1000	9								
		3	8294	10000	25	240000	5,38		250000	680000	250000	5,40
100000	1											
8295	10000		19	190000	5,28		370000	530000	370000	5,57		
	100000		2									
8296	10000		21	210000	5,32		490000	370000	490000	5,69		
	100000	2										
8297	10000	23	220000	5,34		490000	480000	490000	5,69			
	100000	1										
8298	10000	25	250000	5,40		490000	430000	490000	5,69			
	100000	2										

\* Analyses performed according to the COFRAC accreditation



**Appendix 7 - Accuracy profile study: summarized results**

(Food) Category 1		RTE										
(Food) Type 1		Cooked delicatessen: liver pâté										
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7504-7508	Liver pâté	1	300	360	310	270	230	400	270	310	330	360
7519-7523	Liver pâté	1	210	220	290	220	260	300	360	300	400	280
7509-7513	Liver pâté	2	5100	4800	6400	5500	4700	12000	11000	9100	21000	11000
7524-7528	Liver pâté	2	5900	4900	6200	4800	5900	7800	12000	12000	7800	7800
7514-7518	Liver pâté	3	96000	95000	100000	94000	99000	490000	250000	300000	150000	150000
7529-7523	Liver pâté	3	84000	87000	87000	99000	95000	170000	210000	150000	250000	210000

(Food) Category 2		Meat products										
(Food) Type 2		Raw meat: ground beef										
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7650-7654	Ground beef	1	360	340	360	390	350	360	620	440	730	640
7665-7669	Ground beef	1	470	270	340	350	370	710	250	570	330	580
7655-7659	Ground beef	2	6200	7600	5900	5500	5800	6000	7800	5000	5500	5500
7670-7674	Ground beef	2	6400	5500	5800	5500	5900	6000	11000	7400	7800	15000
7660-7664	Ground beef	3	95000	88000	98000	110000	80000	210000	300000	210000	170000	210000
7675-7679	Ground beef	3	120000	110000	130000	110000	93000	170000	210000	250000	300000	370000

(Food) Category 3		Dairy products										
(Food) Type 3		Milk: pasteurized milk										
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
7750-7754	Pasteurized milk	1	190	320	240	210	170	360	240	400	300	260
7765-7769	Pasteurized milk	1	320	330	330	340	330	300	240	390	640	360
7755-7759	Pasteurized milk	2	3600	3800	4500	4400	3700	6800	11000	6800	6800	3700
7770-7774	Pasteurized milk	2	4900	5100	5000	5000	6000	9100	7800	5500	6000	7800
7760-7764	Pasteurized milk	3	55000	67000	68000	75000	67000	210000	170000	120000	210000	250000
7775-7779	Pasteurized milk	3	97000	110000	100000	93000	94000	170000	250000	170000	170000	250000

(Food) Category 4		Seafood										
(Food) Type 4		Raw fish: fish fillet										
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
8164-8168	Raw fish fillet	1	340	290	260	240	190	460	400	240	280	300
8179-8183	Raw fish fillet	1	370	280	260	250	430	530	360	310	480	420
8169-8173	Raw fish fillet	2	4900	4600	4300	4200	4200	7800	5300	7800	6000	4500
8184-8188	Raw fish fillet	2	4700	5500	4500	4500	4100	4000	7800	4100	5500	4400
8174-8178	Raw fish fillet	3	58000	56000	65000	59000	55000	110000	170000	150000	170000	250000
8189-8193	Raw fish fillet	3	78000	85000	74000	70000	68000	78000	120000	110000	150000	210000

(Food) Category 5			Vegetables									
(Food) Type 5			RTE: grated carrots									
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
8239-8243	Grated carrots	1	260	240	150	200	220	400	280	360	230	360
8254-8258	Grated carrots	1	160	290	180	200	250	200	330	220	160	400
8244-8248	Grated carrots	2	2500	2700	3700	3800	2800	4000	7800	3400	5500	5500
8259-8263	Grated carrots	2	2500	2600	3500	3200	3300	5500	6000	5000	3400	4000
8249-8253	Grated carrots	3	48000	63000	58000	45000	49000	78000	210000	68000	170000	91000
8264-8268	Grated carrots	3	44000	48000	45000	45000	44000	120000	91000	150000	110000	210000

(Food) Category 6			Pet food									
(Food) Type 6			Pâtés: dog pâté									
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
8269-8273	Dog pâté	1	760	810	670	800	760	1100	1400	950	1600	570
8284-8288	Dog pâté	1	790	870	740	940	800	1000	1200	1100	1400	1500
8274-8278	Dog pâté	2	13000	14000	14000	12000	11000	25000	30000	17000	21000	21000
8289-8293	Dog pâté	2	13000	14000	12000	12000	15000	25000	30000	15000	21000	17000
8279-8283	Dog pâté	3	200000	300000	260000	290000	250000	490000	290000	370000	260000	370000
8294-8298	Dog pâté	3	240000	190000	210000	220000	250000	250000	370000	490000	490000	490000

## Appendix 8 - Inclusivity / Exclusivity: raw data (initial validation, and extension study, 2016)

INCLUSIVITY (initial validation)					
Strain		PCA	ISO 16649-2 <sup>♦</sup>	TEMPO EC	
		log cfu/ml	log cfu/ml	log 1	log 2
1.	<i>E.coli</i> 1	8,87	8,89	8,89	9,08
2.	<i>E.coli</i> 17	9,28	8,53	9,48	9,32
3.	<i>E.coli</i> 19	8,76	8,34	9,04	8,89
4.	<i>E.coli</i> 101	9,15	7,81	9,08	8,78
5.	<i>E.coli</i> 108	9,32	8,66	9,04	8,96
6.	<i>E.coli</i> 2B	8,81	8,76	8,96	8,96
7.	<i>E.coli</i> 118	8,89	8,71	9,04	8,96
8.	<i>E.coli</i> 12	9,08	8,88 White colonies	<6	<6
9.	<i>E.coli</i> 121	8,92	8,71	8,61	8,89
10.	<i>E.coli</i> 123	9,30	8,68	9,32	9,32
11.	<i>E.coli</i> 13	9,15	8,70	9,23	9,18
12.	<i>E.coli</i> 14	9,28	8,70	9,08	9,08
13.	<i>E.coli</i> 143	9,20	7,96	8,83	8,83
14.	<i>E.coli</i> 144	9,15	8,76	8,96	8,96
15.	<i>E.coli</i> 21	8,96	8,61	8,87	8,53
16.	<i>E.coli</i> ATCC 43888	8,93	8,04 White colonies	<6	<6
17.	<i>E.coli</i> 70	9,28	8,64	9,23	8,96
18.	<i>E.coli</i> 9	9,23	8,68	9,18	9,04
19.	<i>E.coli</i> 91	9,36	6,65	6,32	6,52
20.	<i>E.coli</i> 94	9,23	8,76	9,23	9,18
21.	<i>E.coli</i> 96	8,77	8,30	8,89	8,96
22.	<i>E.coli</i> 97	9,04	8,64	9,32	9,18
23.	<i>E.coli</i> Ad 217	9,20	8,63	9,32	8,96
24.	<i>E.coli</i> Ad 222	9,20	8,76	9,18	9,04
25.	<i>E.coli</i> Ad 223	9,18	8,71	9,18	9,04
26.	<i>E.coli</i> Ad 228	9,11	8,26	8,89	8,96
27.	<i>E.coli</i> 18	9,91	8,96	8,89	9,23
28.	<i>E.coli</i> CIP 54127	9,15	8,18	9,08	8,83
29.	<i>E.coli</i> CIP 54117	9,28	8,56	9,23	9,04
30.	<i>E.coli</i> CIP 7624	9,26	8,72	9,40	9,32

♦ Analysis performed according to the COFRAC accreditation

INCLUSIVITY (Extension study, 2016)									
n°	Strain	Reference	Origin	PCA		ISO 16649-2 <sup>♦</sup>		TEMPO EC	
				cfu/mL		cfu/mL		cfu/mL	
				rep 1	rep2	rep 1	rep2	rep 1	rep2
1	<i>E.coli</i>	Ad1422	Milk Powder	53	38	37	30	81	90
2	<i>E.coli</i>	Ad 1816	Raw milk cheese	41	48	<1 (44 White)	<1 (27 White)	<1	<1
3	<i>E.coli</i>	Ad1828	Beef meat	54	58	60	50	81	48
4	<i>E.coli</i>	Ad1999	Poultry meat	31	18	<1 (24 White)	<1 (22 White)	<1	<1
5	<i>E.coli</i>	Ad2001	Chicken leg	60	52	55	55	63	71
6	<i>E.coli</i>	Ad1383	Swimming-pool water	52	53	<1 (46 White colonies)	<1 (44 White colonies)	<1	<1
7	<i>E.coli</i>	Ad1389	Sea water	55	56	58	59	63	57
8	<i>E.coli</i>	Ad1399	River water	39	53	41	43	64	52
9	<i>E.coli</i>	Ad1915	Chicken leg	51	52	42	49	71	57
10	<i>E.coli</i> O26	Ad1739	Raw milk cheese	46	54	47	49	63	64
11	<i>E.coli</i> O103	Ad1743	Ground beef	76	74	63	65	73	95
12	<i>E.coli</i> O145	Ad1863	Ground beef	55	70	50	60	63	140
13	<i>E.coli</i> O26	Ad1740	Raw milk cheese	56	57	43	43	43	64
14	<i>E.coli</i> O103	Ad1862	Cheese	57	70	59	55	160	130
15	<i>E.coli</i> O26	Ad1742	Cheese	46	62	53	45	83	83
16	<i>E.coli</i>	142	Liquid egg product	69	63	57	51	75	110
17	<i>E.coli</i>	Ad219	Poultry meat	49	39	49	50	81	44
18	<i>E.coli</i>	Ad234	Veal meat	63	51	55	56	130	63
19	<i>E.coli</i>	Ad241	Poultry meat	44	32	41	32	110	73
20	<i>E.coli</i>	CIP53126	Poultry meat	35	25	24	29	33	36

♦ Analysis performed according to the COFRAC accreditation

EXCLUSIVITY (Initial validation)					
	Strains	PCA	ISO 16649-2 <sup>♦</sup>	TEMPO EC	
		log cfu/ml	log cfu/ml	log 1	log 2
1.	<i>Bacillus subtilis</i> 630	7,78	<6	<4	<4
2.	<i>Citrobacter diversus</i> 140	9,11	<6	<4	<4
3.	<i>Citrobacter freundii</i> 23	8,96	<6	<4	<4
4.	<i>Enterobacter aerogenes</i> CIP 6086	8,45	<6	<4	<4
5.	<i>Enterobacter cloacae</i> 10	8,48	<6	<4	<4
6.	<i>Enterobacter sakazakii</i> D7	9,30	<6	<4	<4
7.	<i>Enterococcus faecalis</i> 25	9,04	<6	<4	<4
8.	<i>Erwinia carotovora</i> CIP 103762	8,62	<6	<4	<4
9.	<i>Escherichia hermanii</i> 395	9,11	<6	<4	<4
10.	<i>Escherichia vulneris</i> 127	8,36	<6	<4	<4
11.	<i>Escherichia vulneris</i> 132	8,32	<6	<4	<4
12.	<i>Hafnia alvei</i> 111	9,04	<6	<4	<4
13.	<i>Klebsiella oxytoca</i> CIP 7932	8,70	<6	<4	<4
14.	<i>Klebsiella pneumoniae</i> 47	8,90	<6	<4	<4
15.	<i>Kluyvera ascorbata</i> CIP 8295T	8,75	<6	<4	<4
16.	<i>Proteus vulgaris</i> 56	8,74	<6	<4	<4
17.	<i>Providencia stuartii</i> 46	8,72	<6	<4	<4
18.	<i>Rhanella aquatilis</i> 69	8,38	<6	<4	<4
19.	<i>Serratia liquefaciens</i> 26	8,81	<6	<4	<4
20.	<i>Shigella flexneri</i> CIP 8248	8,57	<6	<4	<4

EXCLUSIVITY (Extension study, 2016)									
n°	Strain	Reference	Origin	PCA		ISO 16649-2 <sup>♦</sup>		TEMPO EC	
				cfu/mL		cfu/mL		cfu/mL	
				rep 1	rep2	rep 1	rep2	rep 1	rep2
1	<i>Escherichia hermanii</i>	Ad457	Spinach	30 (-7)	36 (-7)	23NC (-7)	24NC (-7)	<1 (-5)	<1 (-5)
2	<i>Escherichia hermanii</i>	Ad458	Liquid egg white	31 (-7)	31 (-7)	26NC (-7)	26NC (-7)	<1 (-5)	<1 (-5)
3	<i>Escherichia hermanii</i>	Ad462	Raw milk	26 (-7)	35 (-7)	24NC (-7)	6NC (-7)	<1 (-5)	<1 (-5)
4	<i>Escherichia hermanii</i>	Ad460	Custard	38 (-7)	41 (-7)	33NC (-7)	40NC (-7)	<1 (-5)	<1 (-5)
5	<i>Escherichia fergusonii</i>	ATCC35469	/	45 (-7)	33 (-7)	33NC (-7)	21NC (-7)	<1 (-5)	<1 (-5)
6	<i>Leclercia adecarboxylata</i>	Ad707	Milk powder	107 (-6)	132 (-6)	88NC (-6)	97NC (-6)	<1 (-5)	<1 (-5)
7	<i>Morganella sp</i>	Ad1699	Salmon	42 (-6)	52 (-6)	36NC (-6)	41NC (-6)	<1 (-5)	<1 (-5)
8	<i>Buttiauxella agrestis</i>	Ad1328	Liquid egg product	10 (-5)	7 (-5)	5NC (-5)	9NC (-5)	<1 (-5)	<1 (-5)
9	<i>Cronobacter sakazakii</i>	Ad2413	Infant formula	47 (-7)	34 (-7)	36NC (-7)	36NC (-7)	<1 (-5)	<1 (-5)
10	<i>Cronobacter malonaticus</i>	E752	Baby food	27 (-7)	37 (-7)	29NC (-7)	32NC (-7)	<1 (-5)	<1 (-5)

NC: non-characteristic colony

♦ Analysis performed according to the COFRAC accreditation

## Appendix 9 - Limit of quantification (LOQ): raw data

Sample N°	Matrix	Alternative method : TEMPO EC					
		Observed value	Value for calculation	Yi	$\bar{Y}$	S <sub>0</sub>	LOQ
7261	Liver pâté	<10	10	1	1	0	0
7262		<10	10	1			
7263		<10	10	1			
7264		<10	10	1			
7265		<10	10	1			
7266		<10	10	1			
7267		<10	10	1			
7268		<10	10	1			
7269		<10	10	1			
7270		<10	10	1			
7687		Pâté for dog	<10	10			
7688	<10		10	1			
7689	<10		10	1			
7690	<10		10	1			
7691	<10		10	1			
7692	<10		10	1			
7693	<10		10	1			
7694	<10		10	1			
7695	<10		10	1			
7696	<10		10	1			
7697	Grated carrots	<10	10	1	1	0	0
7698		<10	10	1			
7699		<10	10	1			
7700		<10	10	1			
7701		<10	10	1			
7702		<10	10	1			
7703		<10	10	1			
7704		<10	10	1			
7705		<10	10	1			
7706		<10	10	1			
7707	Pasteurized milk	<10	10	1	1	0	0
7708		<10	10	1			
7709		<10	10	1			
7710		<10	10	1			
7711		<10	10	1			
7712		<10	10	1			
7713		<10	10	1			
7714		<10	10	1			
7715		<10	10	1			
7716		<10	10	1			

Sample N°	Matrix	Alternative method : TEMPO EC					
		Observed value	Value for calculation	Yi	$\bar{Y}$	S <sub>0</sub>	LOQ
7717	Fish fillet	<10	10	1	1	0	0
7718		<10	10	1			
7719		<10	10	1			
7720		<10	10	1			
7721		<10	10	1			
7722		<10	10	1			
7723		<10	10	1			
7724		<10	10	1			
7725		<10	10	1			
7726		<10	10	1			
7727		Ground beef	<10	10			
7728	<10		10	1			
7729	10		10	1			
7730	<10		10	1			
7731	<10		10	1			
7732	<10		10	1			
7733	<10		10	1			
7734	<10		10	1			
7735	<10		10	1			
7736	<10		10	1			

## Appendix 10 – Artificial contamination of samples (Extension study, 2023)

Analysis date	N° Sample	Product (French name)	Product	Artificial contamination					Category	Type
				Strain	Origin	Injury protocol	Target inoculation level (CFU/g)	Injury measurement Log CFU		
2022	801	Comté AOP 12 mois affinage Lait cru de vache 35%MG Pâte pressée cuite	Raw cow milk cheese	<i>Escherichia coli</i> 94	Cheese	Seeding 72 h at 5±2°C	5000	/	7	a
2022	802	Parmigiano Reggiano lait cru de vache 30% MG Pâte pressée cuite	Raw cow milk cheese	<i>Escherichia coli</i> 121	Cheese	Seeding 72 h at 5±2°C	50000	/	7	a
2022	803	Brie de Meaux au lait cru de vache Pâte molle et croute fleurie	Raw cow milk cheese	<i>Escherichia coli</i> Ad2828	Cheese	Seeding 72 h at 5±2°C	100000	/	7	a
2022	804	Lait cru de vache	Raw cow milk	<i>Escherichia coli</i> 94	Cheese	Seeding 72 h at 5±2°C	1000	/	7	a
2022	805	Lait cru de vache	Raw cow milk	<i>Escherichia coli</i> 121	Cheese	Seeding 72 h at 5±2°C	100	/	7	a
2022	806	Lait cru de vache	Raw cow milk	<i>Escherichia coli</i> Ad2828	Cheese	Seeding 72 h at 5±2°C	500	/	7	a
2022	807	Brie au lait de vache pasteurisé	Pasteurized cow milk cheese	<i>Escherichia coli</i> 97	Cheese	Spiking 8 min at 56°C	10000	0,3	7	c
2022	808	Brie au lait de vache pasteurisé	Pasteurized cow milk cheese	<i>Escherichia coli</i> Ad3032	Cheese	Spiking 8 min at 56°C	300	0,5	7	c
2022	809	Bûche au lait de chèvre pasteurisé	Pasteurized goat milk cheese	<i>Escherichia coli</i> Ad3032	Cheese	Spiking 8 min at 56°C	3000	0,5	7	c
2022	830	Crème glacée à la pistache	Ice cream	<i>Escherichia coli</i> 15	Raw milk	Seeding 15 days at -20°C	300	/	7	c
2022	831	Crème glacée au lait d'amandes	Ice cream	<i>Escherichia coli</i> 15	Raw milk	Seeding 15 days at -20°C	50000	/	7	c
2022	832	Crème glacée à la vanille	Ice cream	<i>Escherichia coli</i> E17	Dairy product	Seeding 15 days at -20°C	3000	/	7	c
2022	833	Poudre de lait 1/2 écrémé	Milk powder	<i>Escherichia coli</i> 119	Raw milk	Seeding lyophilized 15 days at ambient temperature	1000	/	7	b
2022	834	Poudre de lait 1/2 écrémé	Milk powder	<i>Escherichia coli</i> 16	Raw milk	Seeding lyophilized 15 days at ambient temperature	100	/	7	b
2022	835	Poudre de lait infantile sans probiotiques 1er âge	Infant formula without probiotics	<i>Escherichia coli</i> 119	Raw milk	Seeding lyophilized 15 days at ambient temperature	1000	/	7	b
2022	836	Poudre de lait infantile sans probiotiques 1er âge	Infant formula without probiotics	<i>Escherichia coli</i> Ad1422	Infant formula	Seeding lyophilized 15 days at ambient temperature	10000	/	7	b
2022	837	Poudre de lait infantile avec probiotiques 2e âge	Infant formula with probiotics	<i>Escherichia coli</i> Ad1422	Infant formula	Seeding lyophilized 15 days at ambient temperature	5000	/	7	b
2022	838	Poudre de lait infantile avec probiotiques 1er âge	Infant formula with probiotics	<i>Escherichia coli</i> 16	Raw milk	Seeding lyophilized 15 days at ambient temperature	500	/	7	b
2022	1025	Poudre de lait demi-écrémé	Milk powder	<i>Escherichia coli</i> Ad1422	Infant formula	Seeding lyophilized 15 days at ambient temperature	4000	/	7	b
2022	1026	Poudre de lait infantile avec probiotiques	Infant formula with probiotics	<i>Escherichia coli</i> 119	Raw milk	Seeding lyophilized 15 days at ambient temperature	4000	/	7	b
2022	1027	Poudre de lait infantile sans probiotiques	Infant formula without probiotics	<i>Escherichia coli</i> 119	Raw milk	Seeding lyophilized 15 days at ambient temperature	2500	/	7	b
2022	1129	Poudre de lait demi-écrémé	Milk powder	<i>Escherichia coli</i> 118	Raw milk	Spiking 10 min at 56°C	10000	1,1	7	b
2022	1130	Poudre de lait infantile sans probiotiques	Infant formula without probiotics	<i>Escherichia coli</i> 118	Raw milk	Spiking 10 min at 56°C	5000	1,1	7	b
2022	1131	Poudre de lait infantile avec probiotiques	Infant formula with probiotics	<i>Escherichia coli</i> Ad1816	Raw milk cheese	Spiking 10 min at 56°C	1000	0,5	7	b
2022	1505	Fromage au lait cru de brebis (Ossau Iraty) Pâte pressée non cuite	Raw ewe milk cheese	<i>Escherichia coli</i> E22	Dairy product	Seeding 48 h at 5±2°C	1000	/	7	a
2022	1506	Fromage au lait cru de chèvre Pâte mole	Raw goat milk cheese	<i>Escherichia coli</i> Ad2830	Cheese	Seeding 48 h at 5±2°C	1600	/	7	a
2022	1507	Lait cru de chèvre	Raw goat milk	<i>Escherichia coli</i> Ad2627	Dairy product	Seeding 48 h at 5±2°C	30000	/	7	a
2022	1508	Poudre de lait demi-écrémé	Milk powder	<i>Escherichia coli</i> E22	Dairy product	Spiking 15 min at 56°C	1000	0,4	7	b
2022	1509	Poudre de lait infantile avec probiotiques	Infant formula without probiotics	<i>Escherichia coli</i> E22	Dairy product	Spiking 15 min at 56°C	5000	0,4	7	b
2022	1510	Poudre de lait infantile sans probiotiques	Infant formula with probiotics	<i>Escherichia coli</i> Ad2830	Cheese	Spiking 15 min at 56°C	10000	0,5	7	b



Appendix 11 - Relative trueness study: raw data (Extension study, 2023)

	Dilution used for calculation
	MOPS used for first TEMPO dilution

DAIRY PRODUCTS (primary dilution in BPW)																						
Analysis date	N° Sample	Product (French name)	Product	Dilution	Reference method: NF ISO 16649-2*				Alternative method: TEMPO EC 24 h at 44°C ± 1°C						Alternative method after TEMPO EC cards storage 48 h at 48 h at 5°C ± 3°C						Category	Type
					Plate	CFU/g	CFU/g	log CFU/g	d 1/4	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	d 1/4	d 1/40	d 1/400	d 1/4000	Result	log CFU/g		
2022	801	Comté AOP 12 mois affinage Lait cru de vache 35%MG Pâte pressée cuite	Raw cow milk cheese	100	8	800	800	2,90		730	1300		730	2,86		730	1300		730	2,86	7	a
				1000	0			Ne														
2022	802	Parmigiano Reggiano lait cru de vache 30% MG - Pâte pressée cuite	Raw cow milk cheese	1000	68	64545	65000	4,81			50000	93000	93000	4,97			50000	93000	93000	4,97	7	a
				10000	3																	
2022	803	Brie de Meaux au lait cru de vache Pâte molle et croute fleurie	Raw cow milk cheese	10000	18	172727	170000	5,23			250000	190000	190000	5,28			250000	190000	190000	5,28	7	a
				100000	1																	
2022	804	Lait cru de vache	Raw cow milk	100	41	3909	3900	3,59	3700	3700			3700	3,57	3700	3700			3700	3,57	7	a
				1000	2																	
2022	805	Lait cru de vache	Raw cow milk	10	37	364	360	2,56	680	240			680	2,83	680	240			680	2,83	7	a
				100	3																	
2022	806	Lait cru de vache	Raw cow milk	100	22	2273	2300	3,36	2600	2700			2700	3,43	>4900	2700			2700	3,43	7	a
				1000	3																	
2022	807	Brie au lait de vache pasteurisé	Pasteurized cow milk cheese	100	54	5273	5300	3,72		25000	11000		11000	4,04		25000	11000		11000	4,04	7	c
				1000	4																	
2022	808	Brie au lait de vache pasteurisé	Pasteurized cow milk cheese	10	25	245	250	2,40		180	210		180	2,26		180	210		180	2,26	7	c
				100	2																	
2022	809	Bûche au lait de chèvre pasteurisé	Pasteurized goat milk cheese	100	16	1545	1500	3,18		1800	1200		1800	3,26		1800	1200		1800	3,26	7	c
				1000	1																	
2022	830	Crème glacée à la pistache	Ice cream	10	16	173	170	2,23		160	210		160	2,20		160	210		160	2,20	7	c
				100	3																	
2022	831	Crème glacée au lait d'amandes	Ice cream	1000	21	21818	22000	4,34		37000	78000	36000	36000	4,56		37000	78000	36000	36000	4,56	7	c
				10000	3																	
2022	832	Crème glacée à la vanille	Ice cream	100	21	2000	2000	3,30		1500	1600		1500	3,18		1500	1600		1500	3,18	7	c
				1000	1																	
2022	833	Poudre de lait 1/2 écrémé	Milk powder	10	0	0	<10	<1,00		<10	<100		<10	<1,00		<10	<100		<10	<1,00	7	b
				100	0																	
2022	834	Poudre de lait 1/2 écrémé	Milk powder	10	0	0	<10	<1,00		<10	<100		<10	<1,00		<10	<100		<10	<1,00	7	b
				100	0																	
2022	835	Poudre de lait infantile sans probiotiques 1er âge	Infant formula without probiotics	10	0	0	<10	<1,00		<10	<100		<10	<1,00		<10	<100		<10	<1,00	7	b
				100	0																	
2022	836	Poudre de lait infantile sans probiotiques 1er âge	Infant formula without probiotics	100	0	0	<100	<2,00		10	100		10	1,00		21	100		21	1,32	7	b
				1000	0																	
2022	837	Poudre de lait infantile avec probiotiques 2e âge	Infant formula with probiotics	100	4	400	400	2,60		130	730		130	2,11		160	730		160	2,20	7	b
				1000	1			Ne														
2022	838	Poudre de lait infantile avec probiotiques 1er âge	Infant formula with probiotics	10	2	27	27	1,43*		10	<100		10	1,00		10	<100		10	1,00	7	b
				100	1																	

DAIRY PRODUCTS (primary dilution in BPW)

Analysis date	N° Sample	Product (French name)	Product	Dilution	Reference method: NF ISO 16649-2*				Alternative method: TEMPO EC 24 h at 44°C ± 1°C						Alternative method after TEMPO EC cards storage 48 h at 48 h at 5°C ± 3°C						Category	Type
					Plate	CFU/g	CFU/g	log CFU/g	d 1/4	d 1/40	d 1/400	d 1/4000	Result	log CFU/g	d 1/4	d 1/40	d 1/400	d 1/4000	Result	log CFU/g		
2022	1007	Lait cru de brebis	Raw ewe milk	10	0	0	<10	<1,00	<1	<10			<1	0,00	<1	<10			<1	0,00	7	a
				100	0																	
2022	1008	Lait cru de chèvre	Raw goat milk	10	0	0	<10	<1,00	<1	<10			<1	0,00	<1	<10			<1	0,00	7	a
				100	0																	
2022	1009	Fromage au lait cru de brebis (Roquefort) Pâte persillée	Raw ewe milk cheese	10	0	0	<10	<1,00		<10	<100		<10	<1,00		<10	<100	<10	<1,00	7	a	
				100	0																	
2022	1025	Poudre de lait demi-écrémé	Milk powder	10	8	80	80	1,90		10	<100		10	1,00		10	<100	10	1,00	7	b	
				100	0			Ne														
2022	1026	Poudre de lait infantile avec probiotiques	Infant formula with probiotics	10	0	0	<10	<1,00		<10	<100		<10	<1,00		<10	<100	<10	<1,00	7	b	
				100	0																	
2022	1027	Poudre de lait infantile sans probiotiques	Infant formula without probiotics	10	0	0	<10	<1,00		<10	100		<10	<1,00		<10	100	<10	<1,00	7	b	
				100	1																	
2022	1129	Poudre de lait demi-écrémé	Milk powder	10	157	1500	1500	3,18		500	<100		500	2,70		830	<100	830	2,92	7	b	
				100	8																	
2022	1130	Poudre de lait infantile sans probiotiques	Infant formula without probiotics	10	56	600	600	2,78		230	<100		230	2,36		260	<100	260	2,41	7	b	
				100	10																	
2022	1131	Poudre de lait infantile avec probiotiques	Infant formula with probiotics	10	40	418	420	2,62		<10	<100		<10	<1,00		<10	<100	<10	<1,00	7	b	
				100	6																	
2022	1505	Fromage au lait cru de brebis (Ossau Iraty) Pâte pressée non cuite	Raw ewe milk cheese	10	58	555	560	2,75		480	1300		480	2,68		480	1300	480	2,68	7	a	
				100	3																	
2022	1506	Fromage au lait cru de chèvre Pâte molle	Raw goat milk cheese	100	20	2091	2100	3,32		2400	3800		2400	3,38		2400	3800	2400	3,38	7	a	
				1000	3																	
2022	1507	Lait cru de chèvre	Raw goat milk	100	>150	9000	9000	3,95		49000	31000		31000	4,49		49000	31000	31000	4,49	7	a	
				1000	9			N'														
2022	1508	Poudre de lait demi-écrémé	Milk powder	10	7	70	70	1,85		520	330	1000	520	2,72		520	330	1000	520	2,72	7	b
				100	2			Ne														
2022	1509	Poudre de lait infantile avec probiotiques	Infant formula with probiotics	10	32	318	320	2,51		9100	4800	4500	9100	3,96		9100	4800	4500	9100	3,96	7	b
				100	3																	
2022	1510	Poudre de lait infantile sans probiotiques	Infant formula without probiotics	100	44	4364	4400	3,64		140	21000	<1000	21000	4,32		1400	21000	<1000	21000	4,32	7	b
				1000	4																	

Appendix 12 - Accuracy profile study: raw data (Extension study, 2023. Data from 2022)

Matrix	Strain	Level	Sample N°	Reference method: NF ISO 16649-2*					Alternative method: TEMPO EC 24 h at 44°C				
				Dilution	cfu/plate	cfu/g	cfu/g (rounded)	log cfu/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	cfu/g	log cfu/g
Pasteurized cow milk cheese Batch 1 Aerobic mesophilic flora: 3,1 10 <sup>7</sup> CFU/g	<i>Escherichia coli</i> 94	1	875	10	20	182	180	2,26	260	210		260	2,41
				100	0								
			876	10	26	255	260	2,41	280	590		280	2,45
				100	2								
			877	10	32	300	300	2,48	360	100		360	2,56
		100		1									
		878	10	31	282	280	2,45	330	210		330	2,52	
			100	0									
		879	10	32	291	290	2,46	360	330		360	2,56	
			100	0									
		2	880	100	33	3182	3200	3,51	11000	8100		11000	4,04
				1000	2								
			881	100	32	3091	3100	3,49	5000	7100		5000	3,70
				1000	2								
			882	100	37	3636	3600	3,56	11000	4400		11000	4,04
		1000		3									
		3	883	100	28	3182	3200	3,51	3100	5700		3100	3,49
				1000	7								
			884	100	37	3545	3500	3,54	6800	9300		6800	3,83
				1000	2								
			885	1000	53	51818	52000	4,72		120000	140000	140000	5,15
		10000		4									
		886	1000	42	39091	39000	4,59		150000	57000	57000	4,76	
			10000	1									
		887	1000	52	50909	51000	4,71		150000	58000	58000	4,75	
			10000	4									
		888	1000	67	64545	65000	4,81		120000	130000	130000	5,11	
10000	4												
889	1000	75	80000	80000	4,90		120000	83000	83000	4,92			
	10000	13											
Pasteurized cow milk cheese Batch 2 Aerobic mesophilic flora: 2,3 10 <sup>7</sup> CFU/g	<i>Escherichia coli</i> 94	1	890	10	19	191	190	2,28	250	320		250	2,40
				100	2								
			891	10	22	218	220	2,34	280	450		280	2,45
				100	2								
			892	10	9	100	90	1,95 Ne	140	<100		140	2,15
		100		2									
		893	10	15	145	150	2,18	130	<100		130	2,11	
			100	1									
		894	10	10	100	100	2,00	160	210		160	2,20	
			100	1									
		2	895	100	56	5727	5700	3,76	4500	7300		4500	3,65
				1000	7								
			896	100	31	3000	3000	3,48	2500	11000		2500	3,40
				1000	2								
			897	100	24	2545	2500	3,40	4100	4800		4100	3,61
		1000		4									
		898	100	23	2364	2400	3,38	3100	3600		3100	3,49	
			1000	3									
		899	100	25	2364	2400	3,38	4000	2100		4000	3,60	
			1000	1									
		3	900	1000	68	67273	67000	4,83		370000	110000	110000	5,04
				10000	6								
			901	1000	63	62727	63000	4,80		91000	110000	110000	5,04
				10000	6								
			902	1000	82	82727	83000	4,92		170000	93000	93000	4,97
		10000		9									
		903	1000	58	59091	59000	4,77		170000	200000	200000	5,30	
10000	7												
904	1000	73	71818	72000	4,86		110000	81000	81000	4,91			
	10000	6											

**Appendix 13 - Accuracy profile study: summarized results (Extension study, 2023. Data from 2022)**

(Food) Category 1			Dairy product									
(Food) Type 1			Pasteurized cheese									
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
875-879	Pasteurized cow milk cheese	1	180	260	300	280	290	260	280	360	330	360
890-894	Pasteurized cow milk cheese	1	190	220	90	150	100	250	280	140	130	160
880-884	Pasteurized cow milk cheese	2	3200	3100	3600	3200	3500	11000	5000	11000	3100	6800
895-899	Pasteurized cow milk cheese	2	5700	3000	2500	2400	2400	4500	2500	4100	3100	2100
885-889	Pasteurized cow milk cheese	3	52000	39000	51000	65000	80000	140000	57000	58000	130000	83000
900-904	Pasteurized cow milk cheese	3	67000	63000	83000	59000	72000	110000	110000	93000	200000	81000

### Appendix 14 - Inter-laboratory study results (initial validation, 2005)

Laboratory	N° sample	Reference method: ISO 16649-2						Alternative method: TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arrounded)	log ufc/ml	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
A	A5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	A8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	A2	10	8	6	70	e 70	1,85	100	<100	2,00	<2
		100	1	0							
	A6	10	5	5	50	e 50	1,70	45	100	1,65	2,00
		100	1	0							
	A3	10	83	54	659	660	2,82	630	1 000	2,80	3,00
		100	4	4							
	A7	10	53	67	627	630	2,80	570	1 200	2,76	3,08
		100	11	7							
	A1	100	37	52	4 318	4 300	3,63	15 000	11 000	4,18	4,04
		1000	4	2							
A4	100	33	33	3 500	3 500	3,54	6 000	16 000	3,78	4,20	
	1000	7	4								
B	B5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	B8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	B2	10	8	8	80	e 80	1,90	89	210	1,95	2,32
		100	2	0							
	B6	10	5	12	85	e 85	1,93	100	100	2,00	2,00
		100	1	0							
	B3	10	60	50	527	530	2,72	810	1 300	2,91	3,11
		100	2	4							
	B7	10	71	72	736	740	2,87	830	1 200	2,92	3,08
		100	11	8							
	B1	100	52	50	4 727	4 700	3,67	15 000	11 000	4,18	4,04
		1000	1	1							
B4	100	32	43	3 682	3 700	3,57	7 800	5 100	3,89	3,71	
	1000	3	3								

e :estimation

Laboratory	N° sample	Reference method:						Alternative method:			
		ISO 16649-2						TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arounded)	log cfu/g	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
<b>C</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	C5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	C8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	C2	10	8	6	70	e 70	1,85	99	<100	2,00	<2
		100	0	0							
	C6	10	9	6	75	e 75	1,88	59	<100	1,77	<2
		100	0	0							
	C3	10	57	61	595	600	2,78	710	860	2,85	2,93
		100	7	6							
	C7	10	56	62	564	560	2,75	950	730	2,98	2,86
		100	4	2							
	C1	100	36	38	3 364	3400	3,53	12 000	11 000	4,08	4,04
		1000	0	0							
C4	100	58	17	3 409	3 400	3,53	11 000	5 600	4,04	3,75	
	1000	0	0								
<b>D</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	D5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	D8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	D2	10	11	5	80	e 80	1,90	86	<100	1,93	<2
		100	0	0							
	D6	10	5	4	45	e 45	1,65	45	<100	1,65	<2
		100	0	0							
	D3	10	80	67	732	730	2,86	530	550	2,72	2,74
		100	3	11							
	D7	10	67	75	686	690	2,84	900	1100	2,95	3,04
		100	5	4							
	D1	100	43	46	4 364	4 400	3,64	12 000	11 000	4,08	4,04
		1000	2	5							
D4	100	39	46	4 136	4 100	3,61	11 000	8 300	4,04	3,92	
	1000	3	3								

e :estimation

Laboratory	N° sample	Reference method: ISO 16649-2						Alternative method: TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arounded)	log cfu/g	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
<b>E</b>  Aerobic mesophilic flora: 3,8.10 <sup>6</sup> /g	E5	10	6	7	e 65	65	1,81	<10	<100	<1	<2
		100	0	0							
	E8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	E2	10	0	0	<10	<10	<1	32	<10	1,51	<2
		100	0	0							
	E6	10	8	9	85	85	1,93	86	100	1,93	2,00
		100	0	2							
	E3	10	56	56	555	560	2,75	830	570	2,92	2,76
		100	4	6							
	E7	10	61	64	627	630	2,80	710	570	2,85	2,76
		100	6	7							
	E1	100	52	46	4 773	4 800	3,68	9 100	5600	3,96	3,75
		1000	4	3							
E4	100	38	48	4 227	4 200	3,62	6 000	9 300	3,78	3,97	
	1000	5	2								
<b>F</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	F5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	F8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	F2	10	10	6	73	e 73	1,86	68	<100	1,83	<2
		100	0	0							
	F6	10	6	9	68	e 68	1,83	83	210	1,92	2,32
		100	0	0							
	F3	10	57	54	545	550	2,74	690	570	2,84	2,76
		100	4	5							
	F7	10	76	48	609	610	2,79	1 100	890	3,04	2,95
		100	2	8							
	F1	100	78	74	6 955	7 000	3,85	6 700	9 300	3,83	3,97
		1000	0	1							
F4	100	85	68	7 091	7 100	3,85	9 000	1 100	3,95	3,04	
	1000	1	2								

e :estimation

Laboratory	N° sample	Reference method: ISO 16649-2						Alternative method: TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arounded)	log cfu/g	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
<b>G</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	G5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	G8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	G2	10	5	2	35	e 35	1,54	68	<100	1,83	<2
		100	1	1							
	G6	10	9	7	80	e 80	1,90	33	210	1,52	2,32
		100	1	0							
	G3	10	66	65	650	650	2,81	930	570	2,97	2,76
		100	6	6							
	G7	10	71	66	686	690	2,84	570	590	2,76	2,77
		100	7	7							
	G1	100	53	39	4 409	4 400	3,64	12 000	12 000	4,08	4,08
		1000	3	2							
G4	100	31	30	3 000	3 000	3,48	7 800	7 900	3,89	3,90	
	1000	3	2								
<b>H</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	H5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	H8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	H2	10	6	7	65	e 65	1,81	73	330	1,86	2,52
		100	1	1							
	H6	10	5	4	45	e 45	1,65	21	<100	1,32	<2
		100	0	0							
	H3	10	67	55	586	590	2,77	710	1300	2,85	3,11
		100	5	2							
	H7	10	48	47	468	470	2,67	570	590	2,76	2,77
		100	2	6							
	H1	100	56	48	5 045	5 000	3,70	11 000	11 000	4,04	4,04
		1000	5	2							
H4	100	75	68	6 909	6 900	3,84	9 100	7 100	3,96	3,85	
	1000	4	5								

e :estimation



Laboratory	N° sample	Reference method: ISO 16649-2						Alternative method: TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arounded)	log cfu/g	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
I  Aerobic mesophilic flora: 6,6,0.10 <sup>5</sup> /g	I5	10	0	0	0	<10	<1	<10	<100	<1	<2
		100	0	0							
	I8	10	0	0	0	<10	<1	<10	<100	<1	<2
		100	0	0							
	I2	10	5	4	50	e 45	1,65	71	100	1,85	2,00
		100	1	1							
	I6	10	5	8	59	e 65	1,81	71	100	1,85	2,00
		100	0	0							
	I3	10	79	63	695	700	2,85	830	550	2,92	2,74
		100	5	6							
	I7	10	57	47	514	510	2,71	1 200	1 100	3,08	3,04
		100	6	3							
	I1	100	59	51	5 318	5 300	3,72	6 100	20 000	3,79	4,30
		1000	3	4							
I4	100	49	51	4 727	4 700	3,67	7 800	7 300	3,89	3,86	
	1000	3	1								
J  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	J5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	J8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	J2	10	11	8	95	e 95	1,98	100	100	2,00	2,00
		100	0	2							
	J6	10	2	4	30	e 30	1,48	130	100	2,11	2,00
		100	0								
	J3	10	49	56	532	530	2,72	730	1400	2,86	3,15
		100	9	3							
	J7	10	62	52	568	570	2,76	1 100	1 100	3,04	3,04
		100	7	4							
	J1	100	59	79	7 136	7 100	3,85	15 000	11 000	4,18	4,04
		1000	11	8							
J4	100	>150	>150	13 000	13 000	4,11	12 000	14 000	4,08	4,15	
	1000	15	11								

e :estimation

Laboratory	N° sample	Reference method: ISO 16649-2						Alternative method: TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arrounded)	log cfu/g	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
<b>K</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	K5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	K8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	K2	10	7	4	55	e 55	1,74	71	100	1,85	2,00
		100	0	0							
	K6	10	10	4	70	e 70	1,85	33	<100	1,52	<2
		100	0	0							
	K3	10	47	26	355	360	2,56	790	1 100	2,90	3,04
		100	3	2							
	K7	10	45	49	486	490	2,69	810	1 500	2,91	3,18
		100	6	7							
	K1	100	44	51	4 727	4 700	3,67	7 800	19 000	3,89	4,28
		1000	3	6							
K4	100	42	49	4 591	4 600	3,66	6 000	8 100	3,78	3,91	
	1000	5	5								
<b>L</b>  Aerobic mesophilic flora: >3,0.10 <sup>5</sup> /g	L5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	L8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	L2	10	6	3	45	e 45	1,65	89	<100	1,95	<2
		100	2	0							
	L6	10	8	5	65	e 65	1,81	33	210	1,52	2,32
		100	0	1							
	L3	10	36	51	432	430	2,63	930	210	2,97	2,32
		100	4	4							
	L7	10	57	66	623	620	2,79	830	1600	2,92	3,20
		100	7	7							
	L1	100	17	20	1 727	1 700	3,23	7 800	7 300	3,89	3,86
		1000	1	0							
L4	100	26	18	2 273	2 300	3,36	6 000	14 000	3,78	4,15	
	1000	1	5								

e :estimation

Laboratory	N° sample	Reference method: ISO 16649-2*						Alternative method: TEMPO® EC			
		Dilution	cfu/plate a	cfu/plate b	cfu/g	cfu/g (arrounded)	log cfu/g	D 1/40	D1/400	log cfu/g (1/40)	log cfu/g (1/400)
ADRIA  Aerobic mesophilic flora: 1,2.10 <sup>6</sup> /g	AD5	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	AD8	10	0	0	<10	<10	<1	<10	<100	<1	<2
		100	0	0							
	AD2	10	6	7	68	e 68	1,83	130	<100	2,11	<2
		100	1	1							
	AD6	10	5	7	59	e 59	1,77	21	100	1,32	2,00
		100	0	1							
	AD3	10	72	66	645	650	2,81	900	450	2,95	2,65
		100	2	2							
	AD7	10	82	64	736	740	2,87	1 700	890	3,23	2,95
		100	6	10							
	AD1	100	83	86	8 136	8 100	3,91	15 000	9 000	4,18	3,95
		1000	2	8							
	AD4	100	104	69	8 818	8 800	3,94	7 800	12 000	3,89	4,08
		1000	15	6							

e :estimation

\* Analyses performed according to the COFRAC accreditation