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bioMérieux
Chemin de l'Orme
69280 MARCY L'ETOILE
FRANCE

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

ISO 16140 study validation renewal of TEMPO® EB for the enumeration of *Enterobacteriaceae*

Quantitative method

This document includes 44 pages, with 6 appendixes.
Only copies including the totality of this document are authorized.

Competences of the laboratory are certified by COFRAC accreditation for the analyses marked with symbol◆.

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ADRIA DEVELOPPEMENT
Creac'h Gwen - F. 29196 QUIMPER Cedex - Tél. (33) 02.98.10.18.18 - Fax (33) 02.98.10.18.08
E-mail : adria.developpement@adria.tm.fr - Site web : http://www.adria.tm.fr
ASSOCIATION LOI DE 1901 - N° SIRET 306 964 271 00036 - N° EXISTENCE 532900006329 - N°TVA FR45306964271000036

Before comment

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

The technical protocol and the result interpretation were realized according to the EN ISO 16140 and the AFNOR technical rules.

✓ **Company :**	bioMérieux Chemin de l'Orme F-69280 MARCY L'ETOILE
✓ **Expert Laboratory** :	ADRIA Développement ZA Creac'h Gwen F-29196 QUIMPER Cedex
✓ **Studied method** :	**TEMPO ® EB method**
✓ **Validation standard**:	ISO 16140 (October 2003): Food microbiology – Protocol for the validation of alternative methods
✓ **Reference method♦** :	ISO 21528-2: Microbiology of food and animal feeding stuffs -Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count method
✓ **Scope:**	**All food products and pet food, except beverages and feedstuffs**
✓ **Certification organism:**	AFNOR Certification

♦ Analyses performed according to the COFRAC accreditation

1 INTRODUCTION

1.1 Dates of the validation studies

The TEMPO® EB method was validated in December 2006 (certificate number BIO 12/21 – 12/06), for all human foods products and pet foods, excluding beverages and cattle feed. Renewal studies were performed in 2010 and 2014.

1.2 Alternative method

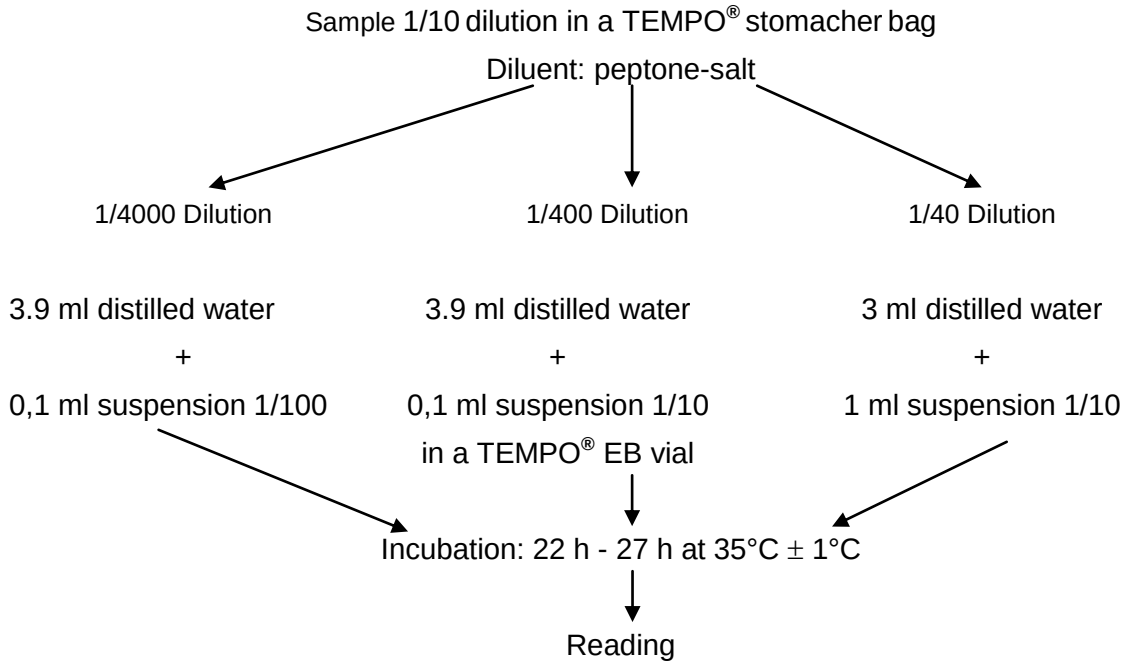
TEMPO® system is an **automated method** associating an **innovative card** with an adapted medium to ensure rapid enumeration of several quality indicators.

The TEMPO® EB test consists of a vial of culture medium and a card, which are specific to this test. The culture medium is inoculated with the sample to be tested and homogeneously transferred by the **TEMPO® Filler** into the card containing 48 wells of three different volumes. The TEMPO® Filler seals the card in order to avoid any risk of contamination during subsequent handling.

The culture medium contains fluorescent pH indicator which, when its pH is neutral, emits a signal detected by the TEMPO® Reader. During incubation, the *Enterobacteriaceae* present in the card assimilate the nutrients in the culture medium, resulting in a decrease of pH and the extinction of the fluorescent signal.

Depending on the number and size of the positive wells, the TEMPO® system deduces the number of *Enterobacteriaceae* present in the original sample according to a calculation based on the MPN (Most Probable Number) method. Card reading, interpretation and reporting are managed by the TEMPO® system after a 22 h incubation at 35°C. TEMPO® EB method allows *Enterobacteriaceae* enumeration.

The general protocol is presented below:



The 1/40 dilution allows 10 to 49 000 cfu/g enumeration
The 1/400 dilution allows 100 to 490 000 cfu/g enumeration
The 1/4000 dilution allows 1 000 to 4 900 000 cfu/g enumeration.

1.3 Reference method

The standard method is the ISO 21528-2: Microbiology of food and animal feeding stuffs -Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count method. The protocol of the method is given in **Appendix 1.**

2 INITIAL VALIDATION AND EXTENSION STUDY RESULTS

2.1 Method comparison study

2.1.1 Linearity

Linearity is the ability of the method when used with a given matrix to give results that are in proportion to the amount of analyte present in the sample, that is an increase in analyte corresponds to a linear or proportional increase in results.

- ***Food matrices and protocols***

The linearity was investigated by mixing serial dilutions of pure culture with 5 food matrices from the following categories: dairy, meat, egg products, seafood and pet food. Five contamination levels were analysed with two repetitions. 50 analyses were performed by the alternative and the standard methods. The contamination levels, the tested samples and the inoculated strains are presented below:

Samples	Strains	Contamination levels (CFU/g)
Ground beef	*Enterobacter cloacae* 128	100 - 500 – 1000 – 5. 10^3 - 1.10^4
Egg product	*Klebsiella pneumoniae 114*	
Milk	*Escherichia coli 94*	
Fish terrine	*Citrobacter freundii 24*	
Pet food	*Hafnia alvei 124*	

During the sample preparation steps, different dilutions were realised according to the contamination levels:

- The samples inoculated with 100 to 5.10^3 CFU/g were analysed by performing a 1/40 dilution.
- The samples inoculated with 500 to 1.10^4 CFU/g were analysed by performing a 1/400 dilution.
- The samples inoculated with 5.10^3 to 1.10^4 CFU/g were analysed by performing a 1/4000 dilution.

Results

Two interpretations were done:

- Interpretation combining all the dilutions:
  Contamination level 1: 1/40
  Contamination level 2: 1/40
  Contamination level 3: 1/400
  Contamination level 4: 1/400
  Contamination level 5: 1/4000
- Interpretation by minimizing dilutions variations:
  Contamination level 1: 1/40
  Contamination levels 2 – 3 – 4 – 5: 1/400

The bi-dimensional graphs are shown in **Appendix 2**.

Statistical interpretations

Statistical interpretation results are shown in the tables below by combining the dilutions or not.

Statistical Interpretation:
P > 5 %: not significant 1 % < P < 5 % :significant P < 1 % : highly significant

- ***Combined dilutions**:*

Matrix	R	Selected regression	Rob.F	Critical value	P%	Correlation coefficient	Regression equation*
Raw ground beef	2.17	OLS1	4.286	5.41	8	0.986	log Alt = 1.114 log Ref - 0.360
Milk	2.70	OLS1	0.000	5.41	100	0.989	log Alt = 1.118log Ref - 0.181
Fish Terrine	8.00	OLS1	1.482	5.41	33	0.991	log Alt = 1.096 log Ref - 0.299
Raw egg product	8.00	OLS1	2.160	5.41	21	0.987	log Alt = 1.045 log Ref - 0.091
Pet food	8.33	OLS1	0.385	5.41	77	0.985	log Alt = 1.004 log Ref - 0.012

** x-axis and y-axis choice depends on the selected regression.*

- ***Dilutions 1/40-1/400**:*

Matrix	R	Selected regression	Rob.F	Critical value	P%	Correlation coefficient	Regression equation*
Raw ground beef	3.33	OLS1	2.887	5.41	14	0.973	log Alt = 1.067 log Ref - 0.137
Milk	0.50	GMFR	0.219	5.41	88	0.999	log Alt = 0.998 log Ref - 0.138
Fish Terrine	8.00	OLS1	1.662	5.41	29	0.991	log Alt = 1.141 log Ref - 0.416
Raw egg product	4.00	OLS1	5.504	5.41	5	0.994	log Alt = 1.058 log Ref - 0.116
Pet food	7.67	OLS1	2.199	5.41	21	0.974	log Alt = 0.965 log Ref - 0.004

** x-axis and y-axis choice depends on the selected regression.*

The regressions are shown in **Appendix 2** (combined dilutions or not).

Discussion

The linearity is accepted in all cases, for all food categories, with P values higher than 5 % and correlation coefficients higher than 0.97.

TEMPO® EB shows satisfying linearity performances.

2.1.2 Relative accuracy

The accuracy is the closeness of agreement between a test result and the accepted reference value.

The bias is the difference between the expectation of the test results and an accepted reference value.

Number and nature of the samples

The food categories and types that were analysed are presented in table 1.

Table 1 – Number and nature of the samples

Food category	Types	Number of samples analysed	Number of results used
Meat products	Raw meat, delicatessen, ready to eat food	22	19
Dairy products	Raw milk, cheese, milk powder	15	11
Fish & Seafood and Fruits & vegetables	Frozen products, salads, ready to eat foods, raw fish and seafood, smoked fish	22	11
Egg products	Egg product, ready to eat foods, pastries	18	12
Pet food	Low and high moisture products, raw meat	15	10

Artificial contamination of the samples

No contamination was realised.

Results

Raw data are given in **Appendix 3**. Samples were analysed in duplicate for each of the two methods.

Table 2

Food category	Contamination scale (log CFU/g)
Meat products	2.08 to 6.52
Dairy products	2.70 to 6.40
Fish & Seafood and Fruits & vegetables	1.40 to 5.98
Egg products	1.52 to 6.08
Pet food	3.36 to 5.69

Bi-dimensional graphs per food category and for all tested samples are presented in **Appendix 4**.

- ***Interpretation***

Table 3

Category	n	R	Regression used	a	t(a)	b	t(b)	Critical T	P%	
									Ordinate at 0	Slope at 1
Meat products	19	3.17	OLS1	0.089	0.439	0.982	0.330	2.110	67	75
Dairy products	11	3.00	OLS1	0.065	0.181	1.025	0.298	2.262	86	77
Egg products	12	1.48	GMFR	-0.346	4.648	1.087	4.886	2.228	0	0
Fish & Seafood and Fruits & vegetables	11	1.83	GMFR	0.130	0.363	0.974	0.249	2.262	72	81
Pet food	10	1.76	GMFR	-0.634	2.190	1.147	2.234	2.306	6	6
All products	63	1.86	OLS1	-0.037	0.318	1.021	0.723	2.000	75	47

The repeatability limits of the alternative and reference methods, as well as the bias D between for both methods, are presented in table 4.

Table 4

Category	Bias D	Alternative method repeatability limit	Reference method repeatability limit
Meat products	0.015	0.558	0.176
Dairy products	0.195	0.264	0.088
Egg products	0.015	0.308	0.455
Fish & Seafood, Fruits & vegetables	-0.015	0.323	0.176
Pet food	0.023	0.440	0.250
All products	0.050	0.382	0.205

The regression straight lines (graph and equation representations) for each food category and for analysed samples are presented in **Appendix 4**.

- ***Discussion***

The bias between both methods is non significant with a low value: + 0.05 log CFU/g.

The reference method repeatability limits show smaller values than the alternative method repeatability limits, except for the “Egg products” category.

The intercept close to 0 and slopes close to 1 are validated by the statistical tests for all categories.

For all food products, the regression straight line is the following:

log (TEMPO EB) = 1.021 log (ref) – 0.037

TEMPO® EB method shows a satisfying relative accuracy.

2.1.3 Detection limit (LOD) and quantification limit (LOQ)

The critical level is the defined as the smallest amount which can be detected (not null), but not quantified as an exact value. Below this value, it cannot be sure that the true value is not null.

The detection limit is defined as being higher than the critical level because it involves a power, the probability 1-β, which has to be well over 50 %, for example 95 %.

The quantification limit is defined as the smallest amount of analyte (that is the lowest actual number of organisms) which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation.

- ***Protocol***

The detection limit of the alternative method was realised with a pure culture of *Escherichia coli* 15.

Three different levels of inoculation were tested, with six replicates per level, i.e. 18 analyses were performed with the alternative method.

The quantification limit was calculated for six independent blank samples.

Results

These data are intrinsic to the alternative method and are presented in the following tables:

Table 5

Level	Positive samples	Standard deviation	Bias
0 CFU/g	0 / 6	/	/
10 CFU/g	5 / 6	0,707	4
50 CFU/g	6 / 6	10.954	10

Table 6

	Formulas	Obtained values
LC	$S_0 + X_0$	5.2
LOD	$S_0 + X_0$	6.3
LOQ	$S_0 + X_0$	11.1

2.1.4 Relative Sensitivity

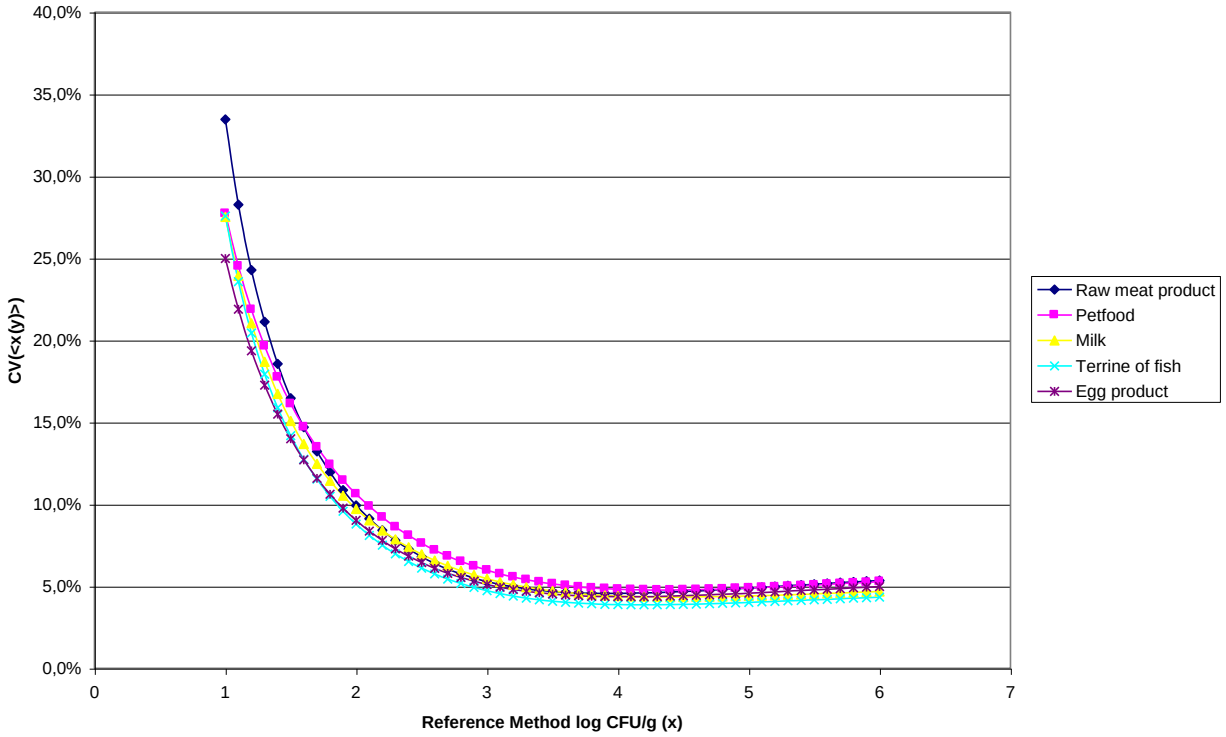
The relative sensitivity is defined as the ability of the alternative method to detect two different amounts of analyte measured by the reference method within a given matrix, at a specified average value, or over the whole measurement range; that is, it is the minimal quantity variation (increase of the analyte concentration x) which gives a significant variation of the measured signal (response y).

These data are intrinsic to the alternative method and are calculated with the linearity study results.

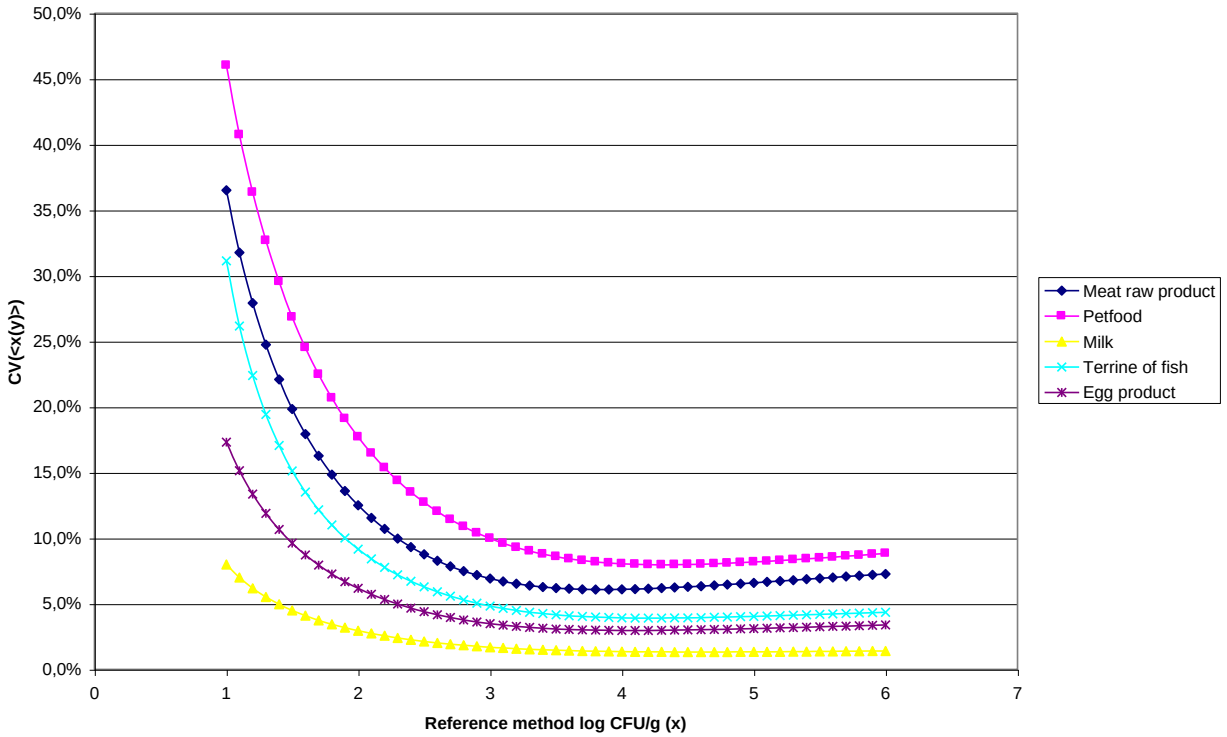
The accuracy patterns are presented in figure 1.

Figure 1 – Accuracy patterns for the different matrices used

Combined dilutions



Dilutions 1/40-1/400



2.1.5 Specificity/Selectivity

The specificity is defined as the degree to which a method is affected (or not) by the other components present in a multi-component sample. That is the ability of a method to measure exactly a given analyte, or its amount, within the sample without interference from non-target components such as a matrix effect, or background noise.

The selectivity is defined as a measure of the degree of non-interference in the presence of non-target analytes. A method is selective if it can be used to detect the analyte under examination, and that a guarantee can be provided that the detected signal can only be a product by that specific analyte.

Trial protocols

After a first growth in BHI broth at appropriated temperatures, decimal dilutions were done and enumerated in duplicate by TEMPO® EB and ISO 21528-2 methods. Bacterial counts were done in parallel with PCA agar in order to verify the inoculation level.

Results

- Inclusivity: 30 positive strains were tested. The results were similar between both methods, except for the *Citrobacter diversus* CIP 8594 strain which was not able to grow on VRBG, but which was enumerated by the TEMPO® EB method.
  The *Rhanella aquatilis* 69 strain was not enumerated by both methods.

- Exclusivity: On 21 tested negative strains, 2 strains of *Xanthomonas maltophilia* gave a positive result with the alternative method and the reference method.

TEMPO® EB method shows satisfying specificity and selectivity.

2.2 Practicability

Workflow study

Time in minutes

Step	Reference method		TEMPO® EB method	
	1 sample	20 samples	1 sample	20 samples
Sampling	2	45	2	45
Diluent, stomach	1	15	2	15
Dilutions	7	67	2	20
Reading	2,5	60	0,45	2
Confirmatory tests	12	195		
Total time	24,5	382	5,45	82
Total time per sample	24,5	19,1	5,45	4,1

The mentioned times do not take into account the medium preparation, which is only required in the reference method. Note that only ready-to-use materials are necessary with TEMPO® EB.

The materials required for one sample analysis are listed below:

- for the realisation of TEMPO® method:
  - 90 ml of diluent,
  - 1 card EB,
  - 1 vial EB,

- for the realisation of NF ISO 21528-2 method:
  - 90 ml of diluent,
  - 2 tubes of 9 ml TS,
  - 6 VRBG Petri Dishes,
  - Nutrient Agar Petri Dishes,
  - Oxidase reagent,
  - tubes of BCP glucose.

One TEMPO® card holder (20 tests EB) is 22,5 cm x 10,5 cm; Note that 120 Petri dishes are needed, i.e. 20 piles of 6 Petri dishes, in ISO 21528-2 method (analysis of 20 samples).

The TEMPO® EB method saves manipulation time, incubation space and materials.

Time results

Step	Reference method	TEMPO® EB method
Sampling, analysis	J0	J0
Reading	J1	J1
Confirmation	J3	/

The time results are significantly reduced with TEMPO® EB: 22 h instead of 72 h.

Traceability among the analyses

TEMPO® Filler and TEMPO® Reader ensure a complete traceability of the following information:

- sample identification,
- test realisation time,
- operator who realised the test,
- material lot references,
- incubation time and time remaining before card reading,
- time card reading,
- number of readings for each card
- operator who realised the reading,
- Results edition in CFU/g,
- possible transfer towards a LIMS (Laboratory Information Management System).

Laboratory maintenance

TEMPO® Reader performances are checked once a month with TEMPO QC kit which is composed of:

- 3 vials TEMPO® QC 8 µM,
- 2 vials TEMPO® QC 20 µM,
- 3 cards TEMPO® QC 8 µM,
- 2 cards TEMPO® QC 20 µM,
- 1 package insert

TEMPO® system offers important economic savings by standardizing the analysis and by minimizing the training time, the volume of wastes and the number of operations. The workflow study demonstrates a 4 to 5 fold reduction of the manipulation time by using TEMPO® EB comparing to the ISO method.

2.3 Inter-laboratory study

2.3.1 Study organisation

12 laboratories participated to this study. Detailed instructions were sent to the collaborators by the expert laboratory.

Pasteurised half-skimmed milk was inoculated with *Escherichia coli* 94 strain, isolated from dairy product.

The inoculation levels targeted were:

- level 0: <10 CFU/ml,
- level 1: 100 – 1 000 CFU/ml,
- level 2: 1 000 – 10 000 CFU/ml,
- level 3: 10 000 – 100 000 CFU/ml.

Each laboratory received eight flasks of 25 ml sample, i.e. two flasks per inoculation level. Furthermore, one non-inoculated sample was added to the package for total viable count (NF ISO 4833 method).

Blind samples were placed in isothermal boxes and delivered to the different laboratories. A probe was added to the package in order to register the temperature profile during the delivery. Samples were shipped in 24 h to laboratories. Sample temperature should be lower or equal to 8°C during delivery, and between 0°C - 8°C at receipt.

The collaborators and the expert laboratory performed the analyses with the alternative and reference methods.

2.3.2 Control of experimental parameters

Strain stability during delivery

In order to evaluate the *Escherichia coli* 94 strain behaviour during transport, bacterial counts were done at different time, i.e. inoculation time, after 24 h and 48 h of storage at 2°C. Results are reported in table 7.

Table 7 - *Escherichia coli* 94 count with the ISO 21528-2 method (in log CFU/ml)

	Level 1		Level 2		Level 3	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
Day 0	100	140	900	850	9 300	7 500
Day 1	100	70	740	570	8 800	7 700
Day 2	70	60	750	750	7 800	5 900

No evolution of the strain was observed after 48 h storage at 4°C.

Results obtained with both methods

Enterobacteriaceae counts obtained by the expert laboratory are presented in table 8.

Table 8 – Expert lab results (in log CFU/g)

Targeted rate (log CFU/g)	Reference method ISO 21528-2		Alternative method TEMPO® EB	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
			D 1/40	D 1/40
< 1	< 1	< 1	< 1	< 1
1 to 2	1.91	1.81	1.77	2.15
2 to 3	2.75	2.74	3.04	2.91
3 to 4	3.88	3.88	4.08	3.96

Targeted contamination levels were reached.

2.3.3 Logistic conditions

Sample temperature at receipt

Measured temperatures at receipt are listed in table 9.

Table 9 – Sample temperature at receipt

Laboratories	Measured temperature on receipt (°C)	Date of sample arrival		Temperature measured by the sensor (°C)
A	3.0	26/09/06	09h10	2.0
B	14.0	27/09/06	09h50	12.0
C	3.1	26/09/06	10h15	0.5
D	1.0	26/09/06	09h00	0.5
E	3.6	26/09/06	08h15	0.0
F	1.0	26/09/06	08h00	0.5
G	1.1	26/09/06	09h30	0.5
H	13.2	27/09/06	11h00	13.5
I	15.1	27/09/06	10h00	14.0
J	17.8	27/09/06	15h30	16.5
K	13.2	26/09/06	13H30	4.5
L	This laboratory did not participate to the study.			
M	3.7	26/09/06	11h00	1.5

Sample temperature during delivery

The packages were received on 06/09/26 by 8 laboratories: labs A, C, D, E, F, G, K and M. The lab K measured a temperature of 13.2°C at receipt while the temperature measured by the probe was 4.5°C.

Four labs received the packages on 06/09/27, with temperature at receipt higher than 8.4°C: labs B, H, I and J.

Growth simulations of the *E. coli* 94 strain were realised with the Sym'Previus[1] software, according to:

- The pH, temperature and a_w cardinal values of *E. coli* strains from the SYM'PREVIUS database,
- The milk physico-chemical parameters (pH and a_w)
- The temperature checked by the probe.

[1] http://www.symprevius.net

For the lab H, the temperature was higher than 8,4°C during a short time. The simulation shows a low bacterial growth, lower than 0,15 Log CFU/g. The lab H data were thus taken into account in the statistical interpretations.

2.3.4 Statistical interpretations and calculations

The calculations were realised according to the amendment number 1 of the ISO 16140 standard (2011):

- ✓ *Accuracy: closeness of agreement between a measurement result and the accepted reference value. Note: Accuracy refers to a combination of trueness and precision*

- ✓ *Trueness: closeness of agreement between the expectation of a measurement result and the accepted reference value. Note: the measure of trueness is usually expressed in terms of bias*

- ✓ *Precision: closeness of agreement between independent measurement results obtained under stipulated conditions. Note: quantitative measures of precision depend critically on the stipulated conditions. Repeatability conditions and reproducibility conditions are particular sets of extreme stipulated conditions*

- ✓ *Repeatability: precision under repeatability conditions*

- ✓ *Repeatability conditions: measurement conditions where independent measurement results are obtained with the same method on identical measurement items in the same laboratory by the same operator using the same equipment within short interval of time.*

- ✓ *Repeatability standard deviation: standard deviation of measurement results obtained under repeatability conditions*

- ✓ *Repeatability limit (r): value less than or equal to which the absolute difference between two measurement results obtained under repeatability conditions is expected to be with a probability of 95%*

- ✓ *Reproducibility: precision under reproducibility conditions*

- ✓ *Reproducibility conditions: measurement conditions where measurement results are obtained with the same method on identical measurement items in different laboratories with different operators using different equipment*

- ✓ *Reproducibility standard deviation: standard deviation of measurement results obtained under reproducibility conditions*

- ✓ *Reproducibility limit (R): value less than or equal to which the absolute difference between two measurement results obtained under reproducibility conditions is expected to be with a probability of 95%*

Summary of the results obtained by both methods

A results summary is presented in table 10.

Table 10 - Results summary of the ISO 21528-2 method (in CFU/g)

Lab.	Level 0				Level 1				Level 2				Level 3			
	Reference method		Atlernative method		Reference method		Atlernative method		Reference method		Atlernative method		Reference method		Atlernative method	
A	<10	<10	<10	<10	110	75	45	86	710	740	730	930	9000	7900	15000	6800
C	<10	<10	<10	<10	85	80	59	10	840	790	730	390	9100	9200	6800	11000
D	<10	<10	<10	<10	75	65	83	120	600	540	710	640	9500	8100	11000	11000
E	<10	<10	<10	<10	65	40	68	83	570	500	520	810	7400	7800	9100	9100
F	<10	<10	<10	<10	80	120	**<10***	100	820	910	530	950	8400	7600	12000	6700
G	<10	<10	<10	<10	120	65	89	57	810	820	360	810	9600	9200	6000	6000
H	<10	<10	<10	<10	65	80	57	83	660	1000	480	400	8000	6100	6000	6800
K	<10	<10	<10	<10	90	150	200	140	1200	1200	2100	1300	14000	12000	30000	15000
M	<10	<10	<10	<10	140	110	59	86	810	930	1400	1100	10000	8300	11000	12000

*** No result was taken into account for the level 1, due to a lack of inoculation**

Scrutiny of the measurement results for consistency

In order to identify other measurement results or laboratories that could be inconsistent, two graphical consistency techniques were realized: the robust Mandel's h-ank-statistics. These graphics are given in **Appendix 5**. All the statistical results are given in **Appendix 6**.

The number of values above the indicator for h and k are given below:

Table 11

Mandel's values	Number of observed values above the indicator	
	Reference method	Alternative method
h > 1 %	/	Lab C Level 1
h > 5 %	Lab D Level 2 Lab E Level 2 Lab K Levels 2 and 3	Lab C Level 1 Lab K Levels 1, 2 and 3
k> 1 %	Lab H Level 2	Lab A Level 3 Lab C Level 1 Lab K Level 3
k > 5 %	Lab H Level 2	Lab A Level 3 Lab C Levels 1 and 3 Lab F Level 3 Lab K Level 3

No inconsistent result was observed. **All the results were used for the statistical analysis.**

- ***Comparison of the trueness and precision characteristics of the reference method and alternative methods***

The statistical values are summarized hereafter:

Table 12

Level	Reference method			Alternative method		
	Median	Repeatability	Reproducibility	Median	Repeatability	Reproducibility
1	1.931	0.135	0.181	1.853	0.150	0.163
2	2.911	0.040	0.061	2.829	0.181	0.216
3	3.943	0.055	0.075	3.959	0.079	0.141

❒ Bias of the alternative method

In order to estimate the bias of the alternative method with respect to the reference method for each level, Dij and t are calculated as described below:

$$Dij = \bar{Y}\,ij, \mathrm{Alt} - \bar{Y}\,ij, \mathrm{Ref}$$

$$t = \frac{\left|\mathrm{median}\ i\ (Dij)\right|}{\sqrt{\pi\ /\ (2\ p)\ \varphi}\ Diff}$$

If t is larger than 2, the alternative method is significantly biased with respect to the reference method.

The values are given in table 13.

Table 13 – Bias values obtained for each level

Level	Bias (D)	t	Interpretation
1	- 0.057	- 0.48	Bias not significant
2	0.056	0.83	Bias not significant
3	0.078	0.60	Bias not significant

All the calculated biases are not significant.

❒ Comparison of the repeatability standard deviations

If the ratio Srj, Alt / Srj, Ref. of the repeatability standard deviations of the alternative method and the reference method is larger than 2, the precision under repeatability conditions of the alternative method is considered to be lower than that of the reference method. If this ratio is smaller than 0.5, the precision under repeatability conditions of the alternative method is considered to be greater than that of the reference method.

The ratio values are given in table 14.

Table 14

Level	Repeatability				
	Reference method		Alternative method		Ratio Alternative / Reference methods
	Repeatability standard deviation	Repeatability	Repeatability standard deviation	Repeatability	
1	0.135	0.377	0.150	0.420	1.113
2	0.040	0.111	0.181	0.507	4.559
3	0.055	0.153	0.079	0.222	1.451

The precision under repeatability conditions of the alternative method is lower than that of the reference method for the inoculation level 2. This precision is greater than that of the reference method for the inoculation levels 1 and 3.

❒ Comparison of the reproducibility standard deviations

If the ratio Srj, Alt / Srj, Ref. of the reproducibility standard deviations of the alternative method and the reference method is larger than 2, the precision under reproducibility conditions of the alternative method is considered to be lower than that of the reference method. If this ratio is smaller than 0,5, the precision under reproducibility conditions of the alternative method is considered to be greater than that of the reference method.

The ratio values are given in table 15.

Table 15

Level	Reproducibility				
	Reference method		Alternative method		Ratio Alternative / Reference methods
	Reproducibility standard deviation	Reproducibility	Reproducibility standard deviation	Reproducibility	
1	0.181	0.506	0.163	0.456	0.900
2	0.061	0.170	0.216	0.606	3.560
3	0.075	0.211	0.141	0.395	1.875

The precision under reproducibility conditions of the alternative method is lower than that of the reference method for the inoculation level 2. This precision is greater than that of the reference method for the inoculation levels 1 and 2.

2.4 Conclusion

The **method comparison study conclusions** are:

- ✓ TEMPO® EB linearity and relative accuracy are satisfying. The repeatability limits of TEMPO® EB method show correct values, comprised between 0,558 and 0,264 log CFU/g.
- ✓ For all products, the bias between both methods is non significant (0,05 log CFU/g).
- ✓ TEMPO® EB method shows satisfying selectivity and specificity performances.
- ✓ TEMPO® system offers important economic savings by:
  - standardizing the analysis
  - minimizing the training time, the volume of wastes and the number of operations.
  - proving directly the final result in 22 h, while 72 h are required with the reference method.

The workflow study demonstrates a 4 to 5 fold reduction of the manipulation time by using TEMPO® EB comparing to the ISO method.

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

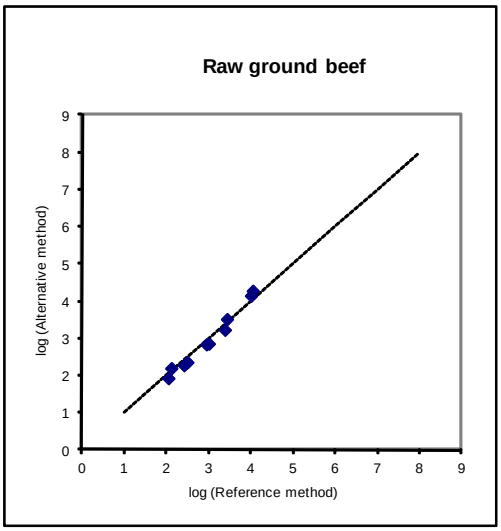
- ✓ Bias observed between both method show low values, between -0.057 and + 0.078; they are all non significant.
- ✓ The repeatability of the TEMPO® EB method is comprised between 0.222 and 0.507; those of the reference method between 0.111 and 0.377.
- ✓ The reproducibility of the TEMPO® EB method is comprised between 0.395 and 0.606; those of the reference method between 0.170 and 0.506.

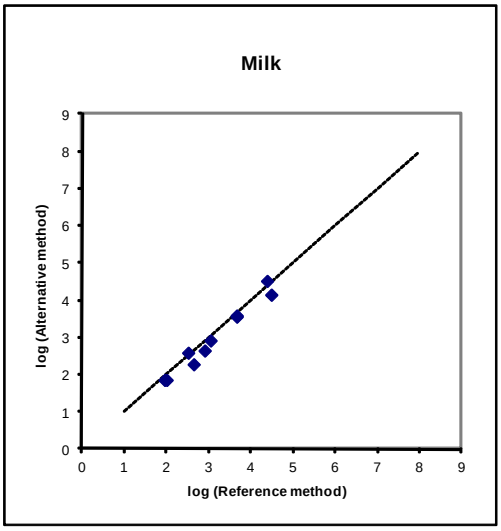
Appendix 1 – ISO 21528-2 reference method:
Microbiology of food and animal feeding stuffs -
Horizontal methods for the detection and enumeration of *Enterobacteriaceae* -
Part 2: Colony-count method

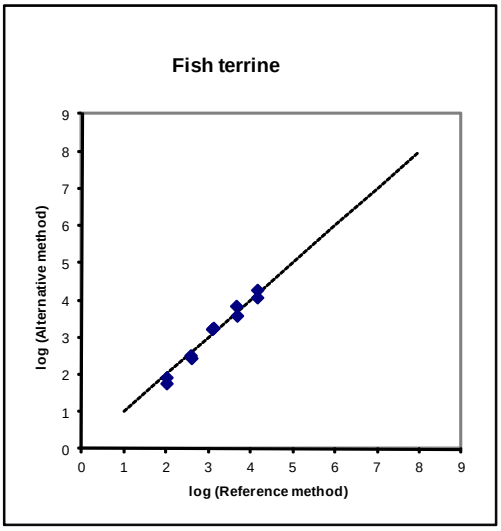
Mother suspension (1/10) in Buffered Peptone Water (solid products
Sample (liquid product)
and decimal dilutions in peptone-salt
↓
1 ml per plate
↓
≈ 10 ml VRBG Agar
↓
Solidification
↓
Double layer (15 ml)
↓
Incubation at 37°C ± 1°C for 24 h ± 2h
↓
Confirmatory tests:
Oxidase, BCP glucose fermentation

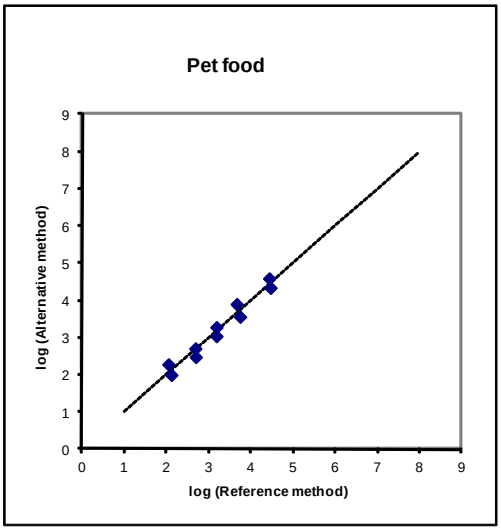
Appendix 2 – Linearity study: bi-dimensional graphs and regression straight lines

Bi-dimensional graphs: combined dilutions analysis

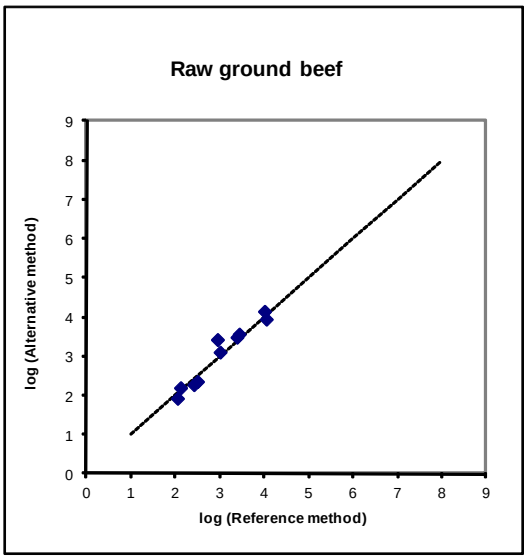


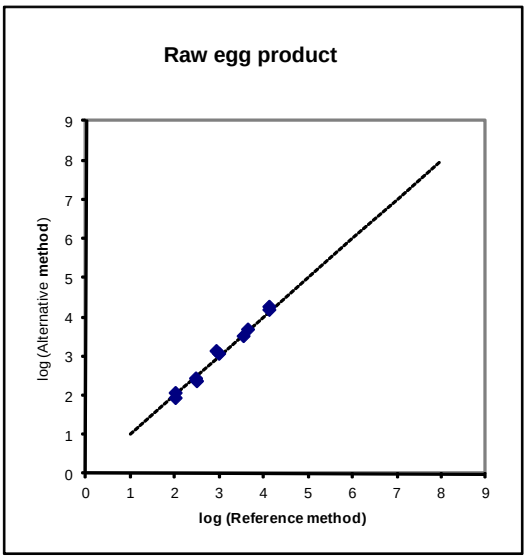


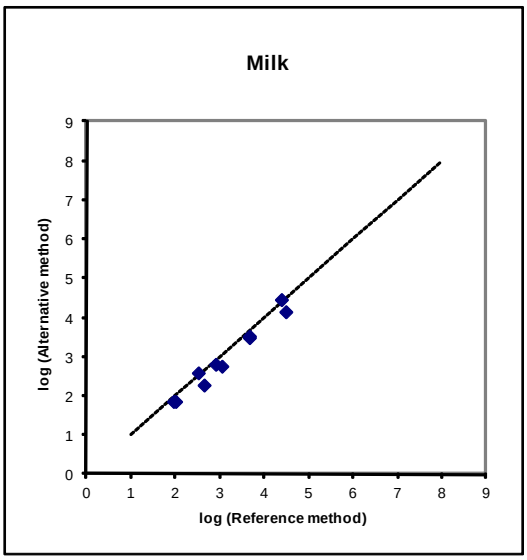


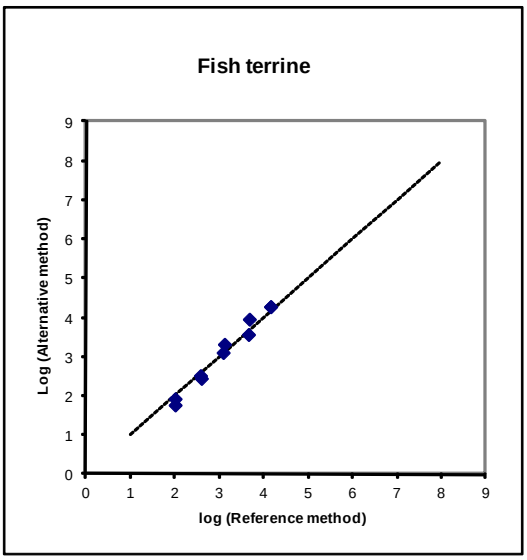


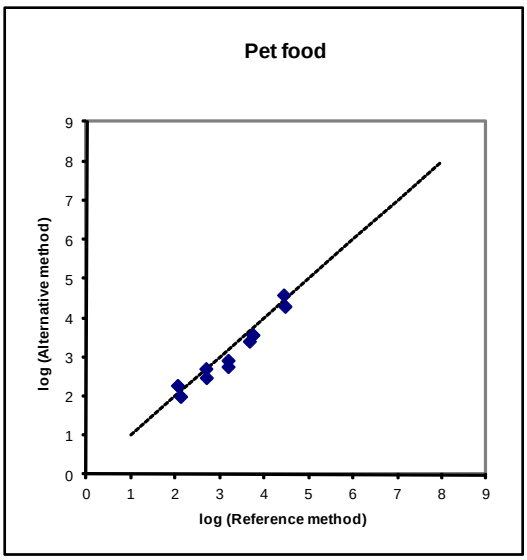
Bi-dimensional graphs : dilutions 1/40-1/400



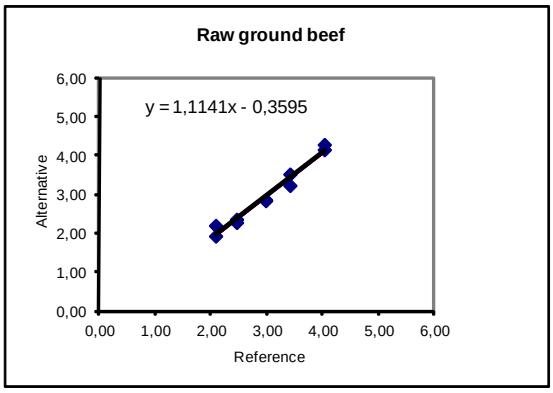


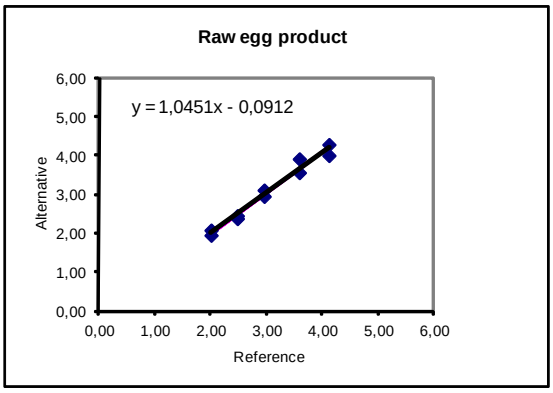


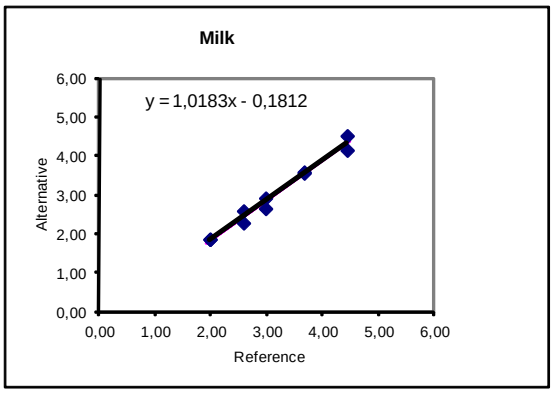


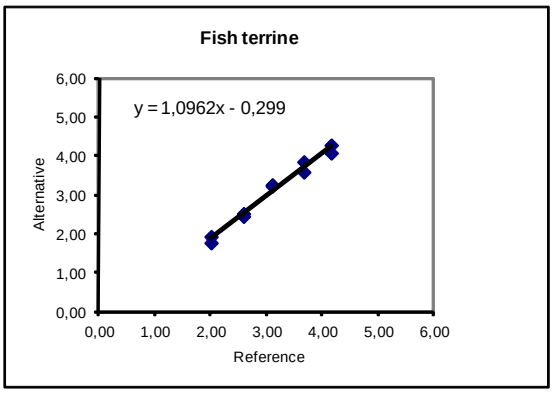


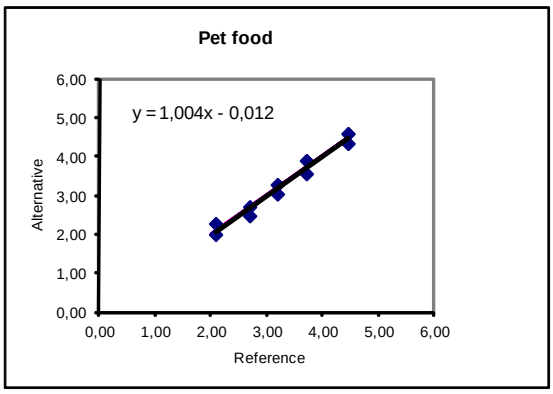
Regression straight lines: combined dilutions analysis



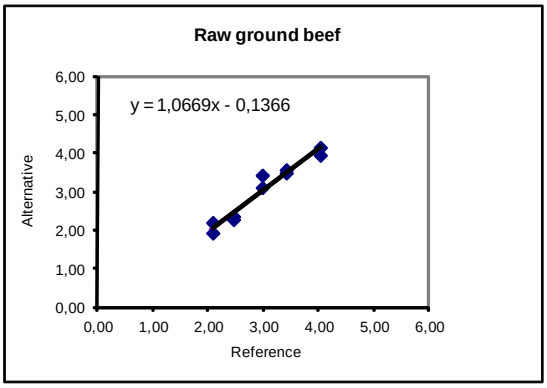


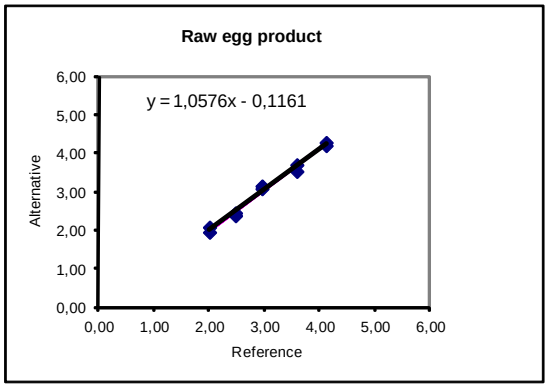


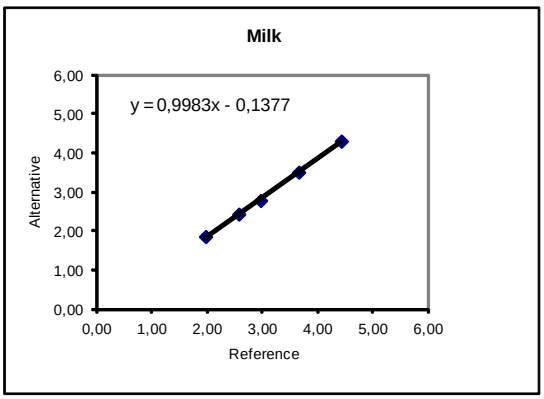


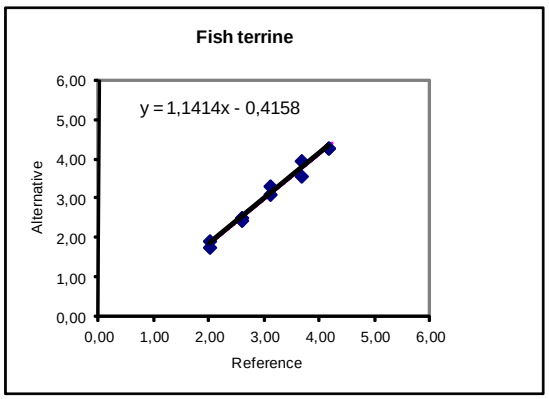


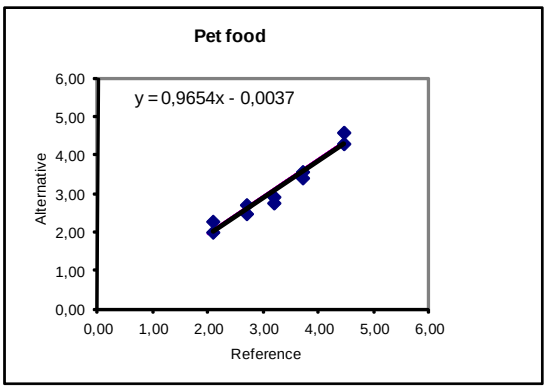
Regression straight lines: dilutions 1/40-1/400











Appendix 3 – Relative accuracy: raw data

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

MEAT PRODUCTS																	
N°	Product (French name)	Dilution	ISO 21528-2◆												TEMPO EB			
			Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation											
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
562	*Sauté de veau*	100	137	125	115	87	137	125	115	87	13000	9900	4.11	4.00	15000	9100	4.18	3.96
		1000	7	11	10	6	7	11	10	6					a	a		
563	*Paupiette de veau*	10	28	20	37	39	28	20	37	39	240	360	2.38	2.56	480	300	2.68	2.48
		100	3	1	1	2	3	1	1	2					a	a		
564	*Paupiette de veau*	10	26	18	25	23	26	18	25	23	220	220	2.34	2.34	180	260	2.26	2.41
		100	4	0	1	0	4	0	1	0					a	a		
565	*Pointes (viande)*	10	Impossible to read												>490000	>490000	>5.69	>5.69
		100													b	b		
566	*Viande blanche*	10	Impossible to read												>490000	>490000	>5.69	>5.69
		100													b	b		
589	*Steak tartare*	10	79	99	86	93	79	99	86	93	870	890	2.94	2.95	1400	390	3.15	2.59
		100	6	8	12	5	6	8	12	5					a	a		
590	*Saucisse*	100	17	23	19	17	17	23	19	17	1800	1800	3.26	3.26	900	1200	2.95	3.08
		1000	0	0	1	3	0	0	1	3					a	a		
591	*Saucisse*	10	35	40	25	42	35	40	25	34	370	280	2.57	2.45	120	210	2.08	2.32
		100	3	4	0	2	3	4	0	2					a	a		
592	*Chair à saucisse*	10	42	62	57	78	42	62	57	78	510	650	2.71	2.81	730	350	2.86	2.54
		100	5	4	3	4	5	4	3	4					a	a		
595	*Porc à l'ananas*	100	Impossible to read												>490000	>490000	>5.69	>5.69
		1000													b	b		
596	*Viande blanche*	1000	4	2	8	7	4	2	8	7	3000	7500	3.48	3.88	6800	6300	3.83	3.80
		10000	0	0	0	0	0	0	0	0	Ne	Ne			b	b		
628	*Viande de porc*	10000	>150	>150	>150	>150	>150	>150	>150	>150	2900000	3300000	6.46	6.52	1700000	1500000	6.23	6.18
		100000	33	24	28	38	33	24	28	38	N'	N'			c	c		

◆ Analysis performed according to the COFRAC accreditation

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

MEAT PRODUCTS																			
N°	Product (French name)	Dilution	ISO 21528-2◆												TEMPO EB			
			Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation											
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
632	Côte de porc	100	61	48	59	76	61	48	59	76	5500	6600	3.74	3.82	15000	9100	4.18	3.96
		1000	7	5	6	5	7	5	6	5					a	a		
633	Andouille	1000	21	22	29	17	21	22	29	17	20000	24000	4.30	4.38	37000	28000	4.57	4.45
		10000	1	1	3	3	1	1	3	3					b	b		
634	Saucisson à l'ail	100	7	5	6	4	7	5	6	4	600	500	2.78	2.70	990	1000	3.00	3.00
		1000	0	0	0	0	0	0	0	0	Ne	Ne			b	b		
635	Saucisse	100	29	40	28	41	29	40	28	41	3400	3300	3.53	3.52	2700	3100	3.43	3.49
		1000	2	3	2	2	2	3	2	2					a	a		
636	Porc au piments	100	47	65	50	64	47	65	50	64	5800	5500	3.76	3.74	7800	3700	3.89	3.57
		1000	7	8	4	2	7	8	4	2					a	a		
638	Merguez	1000	78	65	62	61	78	65	62	61	72000	63000	4.86	4.80	91000	91000	4.96	4.96
		10000	6	10	5	10	6	10	5	10					b	b		
639	Escalope de dinde	100	128	86	122	115	128	86	122	115	11000	12000	4.04	4.08	37000	7900	4.57	3.90
		1000	17	8	19	15	17	8	19	15					b	b		
640	Escalope de poulet	1000	12	9	14	7	12	9	14	7	11000	11000	4.04	4.04	8800	9300	3.94	3.97
		10000	1	0	1	0	1	0	1	0	Ne	Ne			b	b		
641	Steak de cheval	10	>150	>150	>150	50	>150	>150	>150	50	3500	2900	3.54	3.46	9100	4400	3.96	3.64
		100	27	43	18	39	27	43	18	39	N'	N'			a	a		
642	Bouchée à la reine	10	>150	>150	>150	>150	>150	>150	>150	>150	1300	1300	3.11	3.11	1700	1100	3.23	3.04
		100	9	16	14	12	9	16	14	12	N'	N'			a	a		

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

PET FOODS																	
		ISO 21528-2◆												TEMPO EB			
N°	Product (French name)	Dilution	Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation											
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
873	Saucisson pour animaux	100	20	33	22	24	20	33	22	24	2700	2400	3.43	3.38	3100	2400	3.49	3.38
		1000	5	1	0	6	5	1	0	6					a	a		
874	Bouchées pour chien	1000	>150	154	154	>150	>150	154	154	>150	170000	190000	5.23	5.28	250000	250000	5.40	5.40
		10000	15	18	20	17	15	18	20	17	N'	N'			b	b		
875	Viande pour animaux	100	49	67	61	59	29	54	61	59	4100	6000	3.61	3.78	5500	6000	3.74	3.78
		1000	8	4	8	4	5	3	8	4					a	a		
876	Haché pour animaux	10000	36	31	44	37	36	31	44	37	340000	410000	5.53	5.61	300000	490000	5.48	5.69
		100000	5	2	5	4	5	2	5	4					b	b		
887	Graines pour oiseaux	10	/	/	>150	>150	/	/	/	/	6700	11000	3.83	4.04	6700	4400	3.83	3.64
		100	69	53	101	52	55	42	101	122	N'	N'			a	a		
888	Graines pour oiseaux	10	42	23	21	16	0	0	0	0	<10	<10	<1..0	<1.00	<10	<10	<1	<1
		100	1	0	0	1	0	0	0	0					a	a		
939	Saucisson pour chien	100	50	44	46	58	50	44	46	58	4500	5200	3.65	3.72	5000	3100	3.70	3.49
		1000	3	2	5	6	2	2	5	6					a	a		
940	Bouchées pour animaux	100	21	25	39	40	21	25	39	40	2300	3900	3.36	3.59	1200	1200	3.08	3.08
		1000	3	1	4	2	3	1	4	2					a	a		
941	Pâté pour chien	1000	103	78	104	89	103	62	104	89	86000	105000	4.93	5.02	150000	250000	5.18	5.40
		10000	12	13	23	14	12	13	23	14					b	b		
942	Pâté pour chat	10000	20	19	32	24	20	19	32	24	200000	270000	5.30	5.43	300000	300000	5.48	5.48
		100000	1	3	1	2	1	3	1	2					b	b		
943	Croquettes pour chatons	100	106	105	114	111	106	63	68	67	8500	7300	3.93	3.86	6000	11000	3.78	4.04
		1000	13	4	15	10	13	4	15	10					a	a		

◆ Analysis performed according to the COFRAC accreditation

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

SEAFOOD AND VEGETABLES																			
N°	Product (French name)	Dilution	ISO 21528-2◆												TEMPO EB			
			Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation											
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
561	*Epinards à la béchamel*	100	Impossible to read												>490000 b	>490000 b	>5,69	>5,69
		1000																
593	*Piémontaise*	10	29	52	50	50	29	52	50	50	410	510	2.61	2.71	250 a	300 a	2.40	2.48
		100	6	4	4	8	6	4	4	8								
627	*Choux bruxelles*	10	3	1	0	0	3	1	0	0	20	<10	1.30	<1	45 a	44 a	1.65	1.64
		100	0	0	0	0	0	0	0	0	Ne							
629*	*Haricots verts*	10	38	70	37	70	38	70	37	70	530	530	2.72	2.72	86 a	100 a	1.93	2.00
		100	2	6	4	5	2	6	4	5								
630*	*Courgettes*	10	20	17	20	15	20	17	20	15	170	160	2.23	2.20	10 a	<10 a	1.00	<1
		100	1	0	0	1	1	0	0	1								
631	*Poêlée légumes champignons*	10	0	0	0	0	0	0	0	0	<10	<10	<1	<1	<100 b	<100 b	<2.00	<2.00
		100	0	0	0	0	0	0	0	0								
637	*Gambas aux légumes*	100	116	122	86	99	116	122	86	99	12000	9300	4.08	3.97	14000 b	95000 c	4.15	4.98
		1000	13	9	11	8	13	9	11	8								
843	*Terrine de saumon*	10	26	20	16	20	26	20	16	20	220	200	2.34	2.30	120 a	200 a	2.08	2.30
		100	1	1	4	4	1	1	4	4								
844	*Carottes râpées*	10	2	3	3	4	2	3	3	4	25	35	1.40	1.54	45 a	120 a	1.65	2.08
		100	0	0	0	0	0	0	0	0	Ne	Ne						
845	*Céleri rémoulade*	1000	36	31	31	40	36	31	31	40	34000	39000	4.53	4.59	43000 b	55000 a	4.63	4.74
		10000	4	4	7	7	4	4	7	7								
846	*Saumon fumé*	10	3	2	5	1	3	2	5	1	25	30	1.40	1.48	<10 a	27 a	<1	1.43
		100	0	0	0	0	0	0	0	0	Ne	Ne						
877	*Carottes râpées*	10000	>150	>150	>150	>150	/	/	/	/					>4900000 c	>4900000 c	>5.69	>5.69
		100000	>150	>150	>150	>150	/	/	/	/								
878	*Chou rouge*	10000	80	73	123	74	80	44	123	74	630000	950000	5.80	5.98	490000 b	550000 c	5.69	5.74
		100000	11	5	9	6	9	5	7	6								
886	*Terrine de poisson*	10	19	19	20	12	19	19	20	12	180	160	2.26	2.20	440 b	330 a	2.64	2.52
		100	1	1	2	2	1	1	2	2								

* Five colonies observed on VRBG for the reference method which gave negative oxidase tests and positive glucose fermentation, were identified by API 20E galleries. They were not identified as *Enterobacteriaceae*.

◆ Analysis performed according to the COFRAC accreditation

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

SEAFOOD AND VEGETABLES																			
N°	Product (French name)	ISO 21528-2◆												TEMPO EB					
		Dilution	Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation													
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g		
944	*Saumon fumé*	100	103	64	99	86	103	64	99	86	8500	9000	3.93	3.95	9100	7800	3.96	3.89		
		1000	13	8	5	9	13	8	5	9					a	a				
945	*Cocktail de fruits de mer*	10	7	8	5	2	7	8	5	2	75	35	1.88	1.54	210	330	2.32	2.52		
		100	0	4	0	0	0	4	0	0	Ne	Ne			b	a				
946	*Loup de mer*	10	0	0	0	0	0	0	0	0	<10	<10	<1.00	<1.00	<10	<10	<1.00	<1.00		
		100	0	0	0	0	0	0	0	0					a	a				
947	*Julienne de légumes*	100	16	25	27	16	16	25	27	16	2000	2200	3.30	3.34	970	1100	2.99	3.04		
		1000	2	2	2	3	2	2	2	3					a	a				
1014	*Tomates en dés*	100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<2.00	<2.00	32	<10	1.51	<1.00		
		1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000					a	a				
1083	*Thon*	1000	>150	>150	>150	>150									>490000	>490000	>5.69	>5.69		
		10000	>150	>150	>150	>150									b	b				
1084	*Salade provençale*	100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<2.00	<2.00	<10	<10	<1.00	<1.00		
		1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000					a	a				
1085	*Macédoine de légumes*	100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<2.00	<2.00	<10	<10	<1.00	<1.00		
		1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000					a	a				

◆ Analysis performed according to the COFRAC accreditation

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

EGG PRODUCTS																		
N°	Product (French name)	ISO 21528-2◆												TEMPO EB				
		Dilution	Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation											
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
587	Baba au rhum	10	8	11	12	13	8	11	12	13	95	120	1.98	2.08	57	86	1.76	1.93
		100	0	1	1	0	0	1	1	0	Ne	Ne			a	a		
588	Meringue au chocolat	10	50	67	50	53	50	67	50	53	580	510	2.76	2.71	190	270	2.28	2.43
		100	2	8	2	6	2	8	2	6					a	a		
728	Coule d'œuf	1000	>150	>150	>150	>150									>490000	>490000	>5.69	>5.69
		10000	>150	>150	>150	>150									b	b		
826	Gâteau Russe	10	18	20	24	22	18	20	24	22	200	220	2.30	2.34	150	140	2.18	2.15
		100	3	2	2	1	3	2	2	1					a	a		
827	Coule d'œuf	10	26	27	28	19	26	27	28	19	270	230	2.43	2.36	160	150	2.20	2.18
		100	5	1	2	1	5	1	2	1					a	a		
889	Crème d'amande	10	8	6	9	3	3	4	5	2	35	35	1.54	1.54	33	33	1.52	1.52
		100	1	1	0	1	0	0	0	0					a	a		
890	Blanc d'œuf	10	5	5	0	2	3	5	0	1	40	5	1.60	0.70	<10	10	<1.00	1.00
		100	0	0	0	0	0	0	0	0					a	a		
948	Coule d'œuf	10000	>150	>150	>150	>150									>490000	>490000	>5.69	>5.69
		100000	>150	>150	>150	>150									b	b		
949	Omelette	10000	>150	>150	>150	>150									>490000	>490000	>5.70	>5.70
		100000	>150	>150	>150	>150									b	b		
950	Jaune d'œuf	10000	>150	>150	>150	>150									>490000	>490000	>5.71	>5.71
		100000	>150	>150	>150	>150									b	b		
1007	Coule d'œuf	10000	18	44	58	37	11	44	58	22	280000	380000	5.45	5.58	470000	680000	5.67	5.83
		100000	3	4	2	2	3	4	2	1					c	c		
1008	Jaune d'œuf	10000	77	67	113	90	77	67	113	90	780000	1000000	5.89	6.00	820000	1200000	5.91	6.08
		100000	16	11	7	11	16	11	6	11					c	c		
1009	Omelette aux champignons	10000	67	53	60	48	67	53	60	48	590000	550000	5.77	5.74	670000	370000	5.83	5.57
		100000	6	3	9	5	6	3	9	3					c	c		
1012	Œufs brouillés	1000	>150	>150	>150	>150	>150	>150	>150	>150	215000	160000	5.33	5.20	>490000	>490000	>5.69	>5.69
		10000	26	17	22	10	26	17	22	10	N'	N'			b	b		

◆ Analysis performed according to the COFRAC accreditation

a : 1/40 dilution b : 1/400 dilution c : 1/4000 dilution

EGG PRODUCTS																	
N°	Product (French name)	ISO 21528-2◆												TEMPO EB			
		Dilution	Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation											
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
1013	*Blanc d'œuf*	1000	129	128	154	116	129	128	92	116	130000	100000	5.11	5.00	210000	250000	5.32	5.40
		10000	14	12	19	5	14	12	19	3					b	b		
1086	*Omelette sans sel*	1000	15	19	44	27	15	19	44	27	18000	33000	4.26	4.52	49000	17000	4.69	4.23
		10000	4	1	2	1	4	1	0	1					a	a		
1087	*Omelette bio*	1000	40	35	43	32	40	28	34	19	33000	26000	4.52	4.41	30000	49000	4.48	4.69
		10000	3	2	5	1	3	2	3	1					a	a		
1088	*Œufs brouillés*	1000	32	23	44	38	19	23	44	38	20000	39000	4.30	4.59	37000	37000	4.57	4.57
		10000	1	1	3	1	1	1	2	1					b	b		

◆ Analysis performed according to the COFRAC accreditation

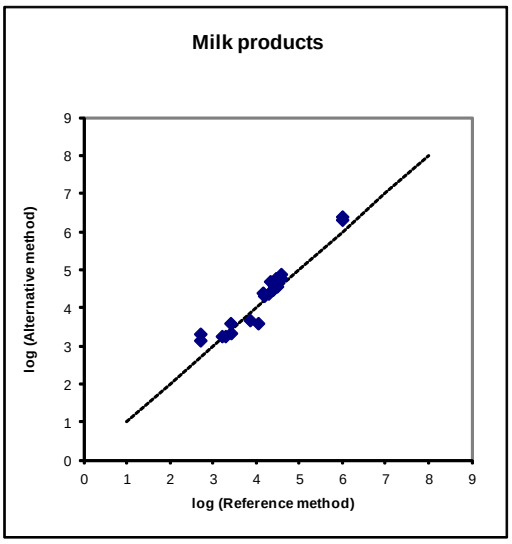
a : dilution 1/40 b : dilution 1/400 c : dilution 1/4000

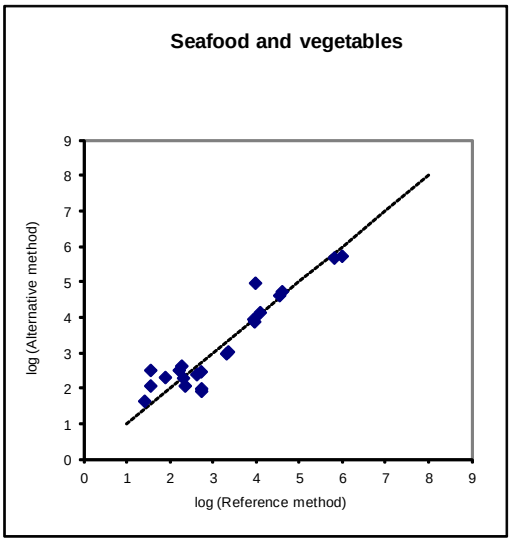
MILK PRODUCTS																		
N°	Produit Product (French name)	ISO 21528-2◆												TEMPO EB				
		Dilution	Replicate 1		Replicate 2		Replicate 1		Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
			Before confirmation				After confirmation													
			cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/plate a	cfu/plate b	cfu/g	cfu/g	log cfu/g	log cfu/g	result	result	log cfu/g	log cfu/g
725	*Lait cru*	100	>150	>150	>150	>150	>150	>150	>150	>150	26000	24000	4.41	4.38	37000	30000	4.57	4.48
		1000	29	22	25	22	29	22	25	22	N'	N'			a	a		
726	*Rocamadour*	100	22	29	25	27	22	29	25	27	2500	2600	3.40	3.41	4000	2200	3.60	3.34
		1000	0	4	4	1	0	4	4	1					a	a		
727	*Mozzarella*	100	>150	>150	>150	>150									370000	370000	5.57	5.57
		1000	>150	>150	>150	>150									b	b		
824	*Lait cru*	100	15	23	17	16	15	23	17	16	1900	1600	3.28	3.20	1800	1800	3.26	3.26
		1000	2	2	2	0	2	2	2	0					a	a		
825	*Mozzarella*	1000	>150	>150	>150	>150	>150	>150	>150	>150	1300000	1000000	6.11	6.00	>490000	>490000	>5.69	>5.69
		10000	1137	132	90	118	137	132	90	118	N'	N'			b	b		
842	*Lait cru*	10000	99	85	110	75	99	85	110	75	970000	970000	5.99	5.99	2100000	2500000	6.32	6.40
		100000	13	16	15	13	13	16	15	13					c	c		
847	*Crottin de chèvre*	10000	>150	>150	>150	>150	>150	>150	>150	>150					>4900000	>4900000	>5.69	>5.69
		100000	>150	>150	>150	>150	>150	>150	>150	>150					c	c		
848	*Crottin de chèvre*	10000	>150	>150	>150	>150	>150	>150	>150	>150					>4900000	>4900000	>5.69	>5.69
		100000	>150	>150	>150	>150	>150	>150	>150	>150					c	c		
879	*Crottin de chèvre*	100	137	150	165	139	137	150	165	139	14000	15000	4.15	4.18	25000	21000	4.40	4.32
		1000	8	11	13	9	8	11	13	9					a	a		
880	*Lait cru*	1000	30	26	39	19	30	26	39	19	27000	30000	4.43	4.48	37000	37000	4.57	4.57
		10000	2	1	6	3	2	1	6	3					a	a		
1000	*Poudre de lait*	1000	8	6	10	12	8	6	10	12	7000	11000	3.85	4.04	4800	4000	3.68	3.60
		10000	0	0	0	1	0	0	0	0	Ne	Ne			b	b		
1001	*Poudre de lait*	1000	1	0	2	2	1	0	1	0	500	500	2.70	2.70	1400	2100	3.15	3.32
		10000	0	0	0	0	0	0	0	0	Ne	Ne			b	c		
1002	*Leerdamer*	1000	36	21	31	21	36	21	31	21	29000	28000	4.46	4.45	37000	60000	4.57	4.78
		10000	5	2	7	2	5	2	7	2					b	b		
1003	*Camembert*	1000	35	32	40	37	35	32	40	37	37000	37000	4.57	4.57	78000	60000	4.89	4.78
		10000	6	8	3	1	6	8	3	1					b	b		
1006	*Reblochon*	1000	26	13	22	14	26	13	22	14	21000	19000	4.32	4.28	50000	24000	4.70	4.38
		10000	4	4	1	4	4	4	1	4					b	b		

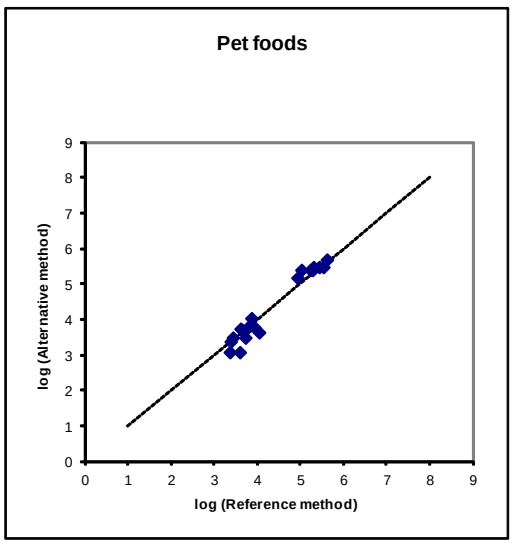
◆ Analysis performed according to the COFRAC accreditation

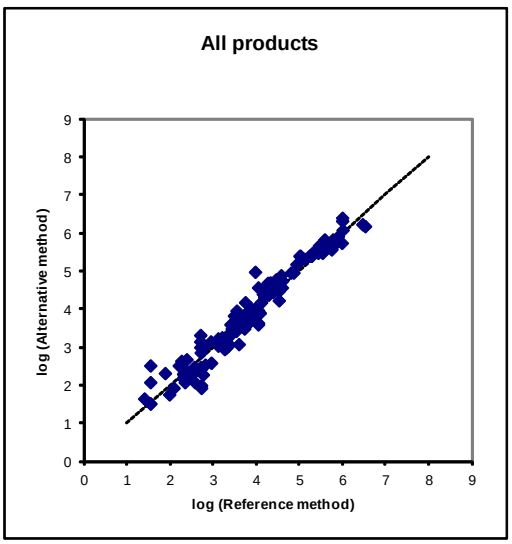
Appendix 4 – Relative accuracy: bi-dimensional graphs and regression straight lines

Bi-dimensional graphs

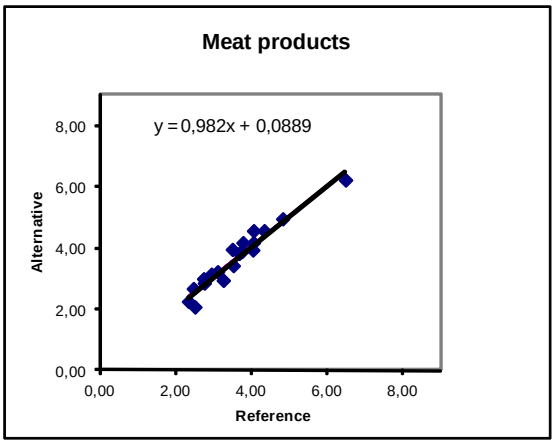


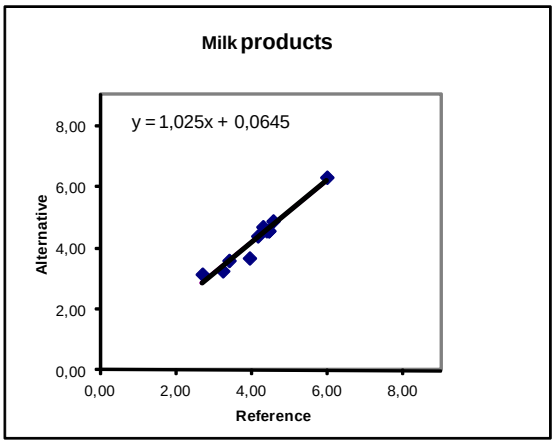


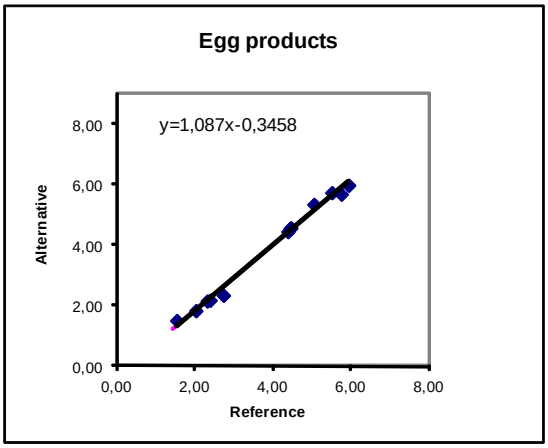


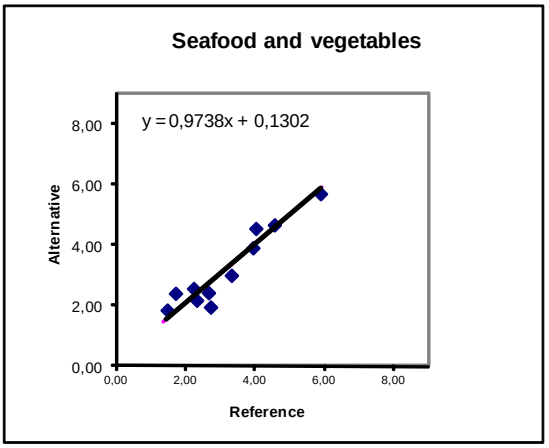


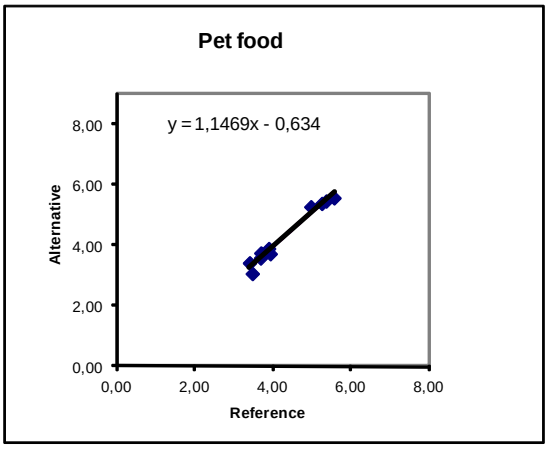
Regression straight lines for each food category and for all matrices

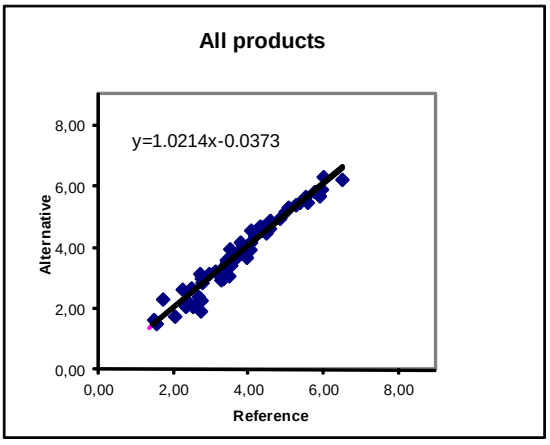






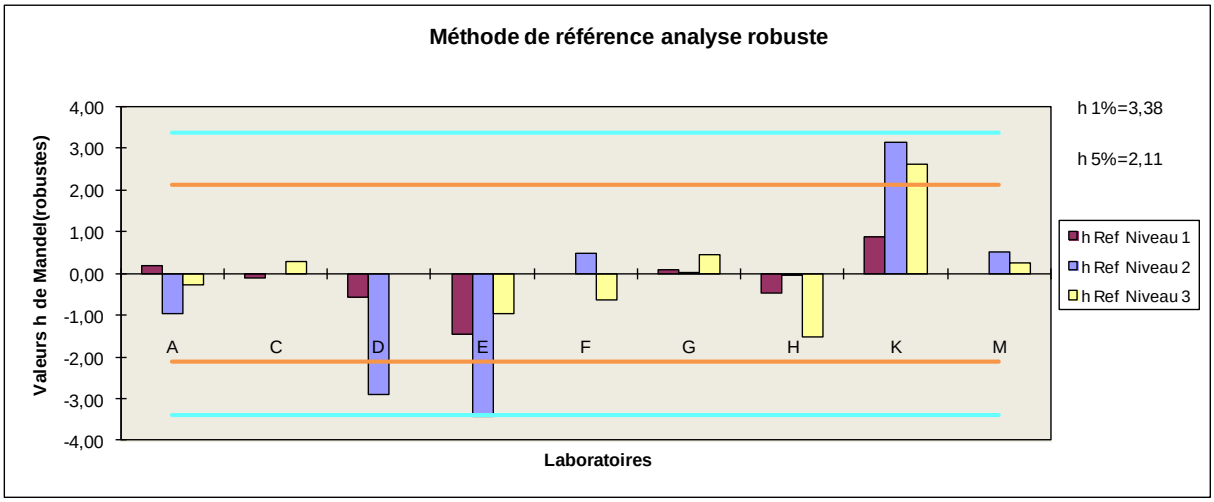


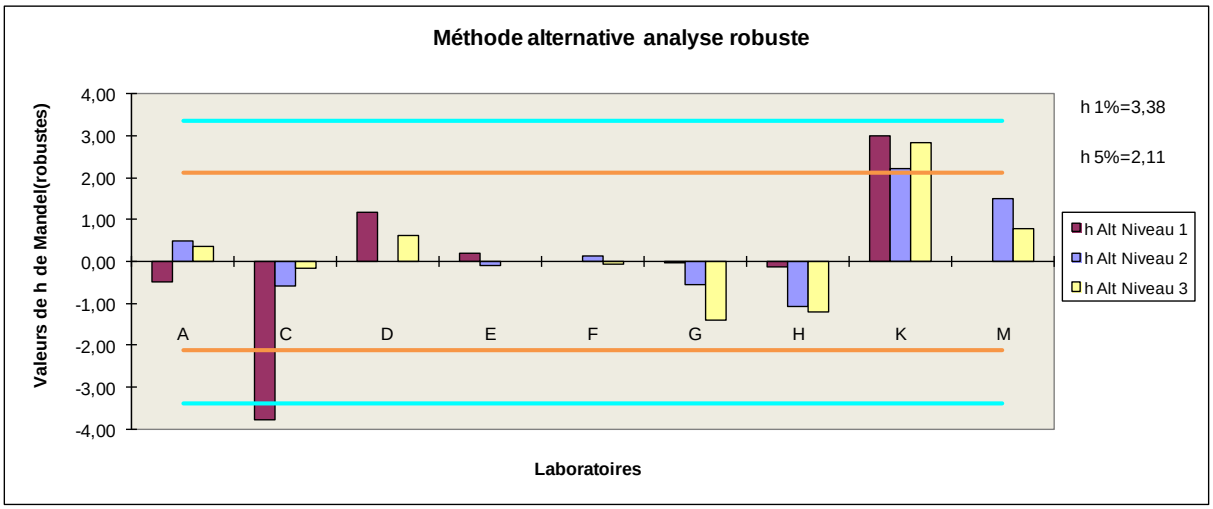




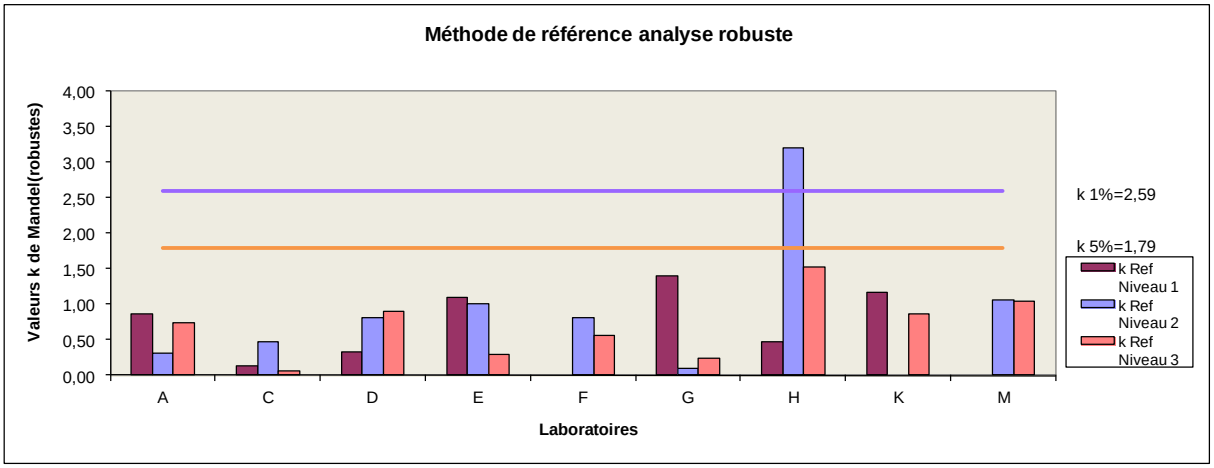
Appendix 5 – Mandel's graphics *(in French)*

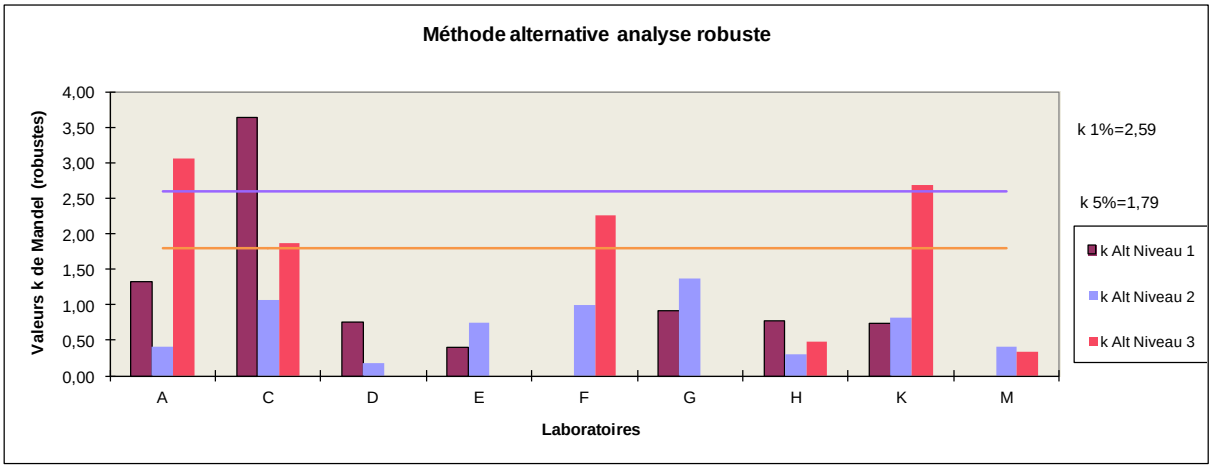
Laboratoires	h Ref Niveau 1	h Ref Niveau 2	h Ref Niveau 3	h Alt Niveau 1	h Alt Niveau 2	h Alt Niveau 3	h5%	h1%	h5%	h1%
A	0,18	-0,95	-0,27	-0,48	0,50	0,35	2,11	3,38	-2,11	-3,38
C	-0,10	0,00	0,28	-3,79	-0,58	-0,17	2,11	3,38	-2,11	-3,38
D	-0,57	-2,91	0,00	1,19	0,00	0,64	2,11	3,38	-2,11	-3,38
E	-1,45	-3,43	-0,97	0,19	-0,09	0,00	2,11	3,38	-2,11	-3,38
F		0,48	-0,63		0,13	-0,05	2,11	3,38	-2,11	-3,38
G	0,10	0,00	0,46	0,00	-0,55	-1,40	2,11	3,38	-2,11	-3,38
H	-0,48	-0,02	-1,53	-0,12	-1,07	-1,19	2,11	3,38	-2,11	-3,38
K	0,87	3,14	2,62	3,01	2,23	2,84	2,11	3,38	-2,11	-3,38
M	1,06	0,51	0,25	0,00	1,52	0,78	2,11	3,38	-2,11	-3,38





Laboratoire	k Ref Niveau 1	k Ref Niveau 2	k Ref Niveau 3	k Alt Niveau 1	k Alt Niveau 2	k Alt Niveau 3	k1%	k5%
A	0,87	0,32	0,73	1,33	0,41	3,07	2,59	1,79
C	0,14	0,47	0,06	3,64	1,06	1,86	2,59	1,79
D	0,33	0,81	0,90	0,76	0,18	0,00	2,59	1,79
E	1,11	1,01	0,30	0,41	0,75	0,00	2,59	1,79
F		0,80	0,56		0,99	2,26	2,59	1,79
G	1,40	0,09	0,24	0,91	1,37	0,00	2,59	1,79
H	0,47	3,21	1,53	0,77	0,31	0,49	2,59	1,79
K	1,16	0,00	0,87	0,73	0,81	2,69	2,59	1,79
M	0,55	1,07	1,05	0,77	0,41	0,34	2,59	1,79





Appendix 6 – Statistical results *(in French)*

Niveau 1

Nombre de laboratoires(p) 8

Laboratoires	Niveau 1 Ref		Moyenne	si	Deviation		h	k
A	2,041	1,875	1,958	0,12	0,083	-0,083	0,176	0,873
C	1,929	1,903	1,916	0,02	0,013	-0,013	-0,097	0,138
D	1,875	1,813	1,844	0,04	0,031	-0,031	-0,567	0,326
E	1,813	1,602	1,707	0,15	0,105	-0,105	-1,455	1,107
G	2,079	1,813	1,946	0,19	0,133	-0,133	0,097	1,397
H	1,813	1,903	1,858	0,06	-0,045	0,045	-0,476	0,473
K	1,954	2,176	2,065	0,16	-0,111	0,111	0,872	1,164
M	2,146	2,041	2,094	0,07	0,052	-0,052	1,058	0,550

	m	1,931151
	Cn(2p)	1,795
	Cn(p)	1,5064
n=2*p	n	16
	f	9
	l	36
n=p	n	8
	f	5
	l	10
	Qn(2p)	0,053059
	Qn(p)	0,1020600
	Qintra	0,09527
	Qinter	0,15374
	Sr(Ecart-type de répétabilité)	0,1347
	RSDr(coefficient de variation de re	6,98%
	r(Limite de répétabilité)	0,377
	SL^2(Ecart-type interlaboratoire)	0,01456
	SR(Ecart-type de reproductibilité)	0,1809
	RSDR(variation coefficient of repr	0,094
	R(Limite de reproductibilité)	0,506

Laboratoires	Niveau 1 Alt		Moyenne	si	Deviation		h	k	D
A	1,653	1,934	1,794	0,20	-0,141	0,141	-0,476	1,327	-0,164
C	1,771	1,000	1,385	0,55	0,385	-0,385	-3,785	3,636	-0,531
D	1,919	2,079	1,999	0,11	-0,080	0,080	1,187	0,755	0,155
E	1,833	1,919	1,876	0,06	-0,043	0,043	0,187	0,408	0,168
G	1,949	1,756	1,853	0,14	0,097	-0,097	0,000	0,913	-0,093
H	1,756	1,919	1,837	0,12	-0,082	0,082	-0,123	0,770	-0,021
K	2,301	2,146	2,224	0,11	0,077	-0,077	3,005	0,731	0,158
M	1,771	1,934	1,853	0,12	-0,082	0,082	0,000	0,772	-0,241

	m	1,85265
	Cn(2p)	1,795
	Cn(p)	1,5064
n=2*p	n	16
	f	9
	l	36
n=p	n	8
	f	5
	l	10
	Qn(2p)	0,0590414
	Qn(p)	0,0819380
	Qintra	0,10601
	Qinter	0,12343
	Sr	0,1499
	RSDr	8,09%
	r	0,420
	SL^2	0,00400
	SR	0,1627
	RSDR	0,088
	R	0,456

Exactitude	
D1	-0,0570
Cp	1,5064
Qn(D)	0,178937
Qdiff	0,2695463
t	-0,48

Niveau 2

Nombre de laboratoires(p) 9

Laboratoires	Niveau 2 Ref		Moyenne	si	Deviation		h	k
A	2,851	2,869	2,860	0,01	-0,009	0,009	-0,947	0,320
C	2,924	2,898	2,911	0,02	0,013	-0,013	0,000	0,474
D	2,778	2,732	2,755	0,03	0,023	-0,023	-2,907	0,814
E	2,756	2,699	2,727	0,04	0,028	-0,028	-3,427	1,013
F	2,914	2,959	2,936	0,03	-0,023	0,023	0,476	0,805
G	2,908	2,914	2,911	0,00	-0,003	0,003	0,004	0,095
H	2,820	3,000	2,910	0,13	-0,090	0,090	-0,022	3,212
K	3,079	3,079	3,079	0,00	0,000	0,000	3,142	0,000
M	2,908	2,968	2,938	0,04	-0,030	0,030	0,514	1,068

	m	2,91095
	Cn(2p)	2,062
	Cn(p)	1,9228
n=2*p	n	18
	f	10
	l	40
n=p	n	9
	f	5
	l	10
	Qn(2p)	0,013627
	Qn(p)	0,0278501
	Qintra	0,02809
	Qinter	0,05355
	Sr(Ecart-type de répétabilité)	0,0397
	RSDr(coefficient de variation de répétabilité	1,36%
	r(Limite de répétabilité)	0,111
	SL^2(Ecart-type interlaboratoire)	0,00208
	SR(Ecart-type de reproductibilité)	0,0608
	RSDR(variation coefficient of reproductibilit	0,021
	R(Limite de reproductibilité)	0,170

Laboratoires	Niveau 2 Alt		Moyenne	si	Deviation		h	k	D
A	2,863	2,968	2,916	0,07	-0,053	0,053	0,500	0,411	0,06
C	2,863	2,591	2,727	0,19	0,136	-0,136	-0,582	1,063	-0,18
D	2,851	2,806	2,829	0,03	0,023	-0,023	0,000	0,176	0,07
E	2,716	2,908	2,812	0,14	-0,096	0,096	-0,094	0,751	0,08
F	2,724	2,978	2,851	0,18	-0,127	0,127	0,128	0,989	-0,09
G	2,556	2,908	2,732	0,25	-0,176	0,176	-0,552	1,375	-0,18
H	2,681	2,602	2,642	0,06	0,040	-0,040	-1,072	0,309	-0,27
K	3,322	3,114	3,218	0,15	0,104	-0,104	2,232	0,813	0,14
M	3,146	3,041	3,094	0,07	0,052	-0,052	1,519	0,409	0,16

	m	2,82872
	Cn(2p)	2,062
	Cn(p)	1,9228
n=2*p	n	18
	f	10
	l	40
n=p	n	9
	f	5
	l	10
	Qn(2p)	0,062130
	Qn(p)	0,0907431
	Qintra	0,12808
	Qinter	0,17448
	Sr(Ecart-type de répé	0,1811
	RSDr(coefficient de v	6,40%
	r(Limite de répétabili	0,507
	SL^2	0,01404
	SR	0,2164
	RSDR	0,077
	R	0,606

Exactitude

D1	0,0557
Cp	1,9228
Qn(D)	0,083242
Qdiff	0,160058
t	0,83

Niveau 3

Nombre de laboratoires(p) 9

Laboratoires	Niveau 3 Ref		Moyenne	si	Deviation		h	k
A	3,954	3,898	3,926	0,04	0,028	-0,028	-0,266	0,733
C	3,959	3,964	3,961	0,00	-0,002	0,002	0,283	0,061
D	3,978	3,908	3,943	0,05	0,035	-0,035	0,000	0,897
E	3,869	3,892	3,881	0,02	-0,011	0,011	-0,966	0,296
F	3,924	3,881	3,903	0,03	0,022	-0,022	-0,628	0,563
G	3,982	3,964	3,973	0,01	0,009	-0,009	0,463	0,239
H	3,903	3,785	3,844	0,08	0,059	-0,059	-1,531	1,525
K	4,146	4,079	4,113	0,05	0,033	-0,033	2,624	0,867
M	4,000	3,919	3,960	0,06	0,040	-0,040	0,254	1,048

Laboratoires	Niveau 3 Alt		Moyenne	si	Deviation		h	k	D
A	4,176	3,833	4,004	0,24	0,172	-0,172	0,349	3,066	0,078
C	3,833	4,041	3,937	0,15	-0,104	0,104	-0,171	1,864	-0,024
D	4,041	4,041	4,041	0,00	0,000	0,000	0,636	0,000	0,098
E	3,959	3,959	3,959	0,00	0,000	0,000	0,000	0,000	0,078
F	4,079	3,826	3,953	0,18	0,127	-0,127	-0,050	2,259	0,050
G	3,778	3,778	3,778	0,00	0,000	0,000	-1,397	0,000	-0,195
H	3,778	3,833	3,805	0,04	-0,027	0,027	-1,187	0,485	-0,039
K	4,477	4,176	4,327	0,21	0,151	-0,151	2,838	2,686	0,214
M	4,041	4,079	4,060	0,03	-0,019	0,019	0,782	0,337	0,101

	m	3,9431
	Cn(2p)	2,062
	Cn(p)	1,9228
n=2*p	n	18
	f	10
	l	40
n=p	n	9
	f	5
	l	10
	Qn(2p)	0,018728
	Qn(p)	0,0336042
	Qintra	0,03861
	Qinter	0,06461
	Sr(Ecart-type de répétabilité)	0,0546
	RSDr(coefficient de variation de répétabilité)	1,38%
	r(Limite de répétabilité)	0,153
	SL^2(Ecart-type interlaboratoire)	0,00268
	SR(Ecart-type de reproductibilité)	0,0753
	RSDR(coefficient de variation de reproductibilité)	0,019
	R(Limite de reproductibilité)	0,211

	m	3,9590
	Cn(2p)	2,062
	Cn(p)	1,9228
n=2*p	n	18
	f	10
	l	40
n=p	n	9
	f	5
	l	10
	Qn(2p)	0,0271788
	Qn(p)	0,0673493
	Qintra	0,05603
	Qinter	0,12950
	Sr(Ecart-type de répétabilité)	0,0792
	RSDr(coefficient de variation de répétabilité	2,00%
	r(Limite de répétabilité)	0,222
	SL^2	0,01363
	SR	0,1411
	RSDR	0,036
	R	0,395

		Exactitude
	D1	0,0784
n=p	Cp	1,9228
	Qn(D)	0,163870
	Qdiff	0,315089
	t	0,60